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STUDIES ON EXPERIMENTAL PNEUMONIA.

III. Spontaneous Pneumonia in Monkeys.

By FRANCIS G. BLAKE, M.D., and RUSSELL L. CECIL, M.D.

(*From the Bacteriological Laboratories of the Army Medical School, Washington.*)

Plates 53 to 64.

(Received for publication, January 23, 1920.)

During the course of the investigation on experimental pneumonia reported in preceding papers[1,2] a considerable amount of spontaneous pneumonia occurred among the stock monkeys. Since this pneumonia was caused in large part by pneumococci, it seemed desirable to study it for the purpose of comparison with pneumonia experimentally produced. Accordingly, autopsies were performed in all fatal cases and cultures were made from the heart's blood, lungs, and bronchi. In a few instances clinical study of the course of the spontaneous disease was carried out, the same methods being applied that were used in the study of experimental pneumonia. The spontaneous pneumonia also presented certain epidemiological features of considerable interest.

Epidemiology.

The monkeys among which spontaneous pneumonia occurred came in three lots at monthly intervals directly from the Philippine Islands to the Army Medical School in Washington. Experience has shown that the transportation of monkeys is usually attended by a considerable mortality *en route*. Every possible care, therefore, was taken to see that the monkeys were properly handled during their journey, and with the exception of one lot the majority arrived in good condition. Upon their arrival those that appeared healthy were placed in a large, well lighted, and well ventilated cage. Those that appeared sick were placed in small individual cages. Since a

[1] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

[2] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 445.

considerable amount of spontaneous pneumonia had occurred among the monkeys of the second lot at the time the third lot arrived, the monkeys of the third lot were placed in pairs in small cages in another room and were not allowed to come into contact with monkeys of the second lot until the outbreak of spontaneous pneumonia had subsided.

The number of monkeys in each lot, the date of their arrival, and the number of recognized cases of pneumonia occurring in each lot are shown in Table I.

The high incidence of pneumonia in Lots 1 and 2, as compared with its total absence among monkeys of Lot 3, is striking. It is of further interest that five of the cases of pneumonia occurring among monkeys of Lot 1 developed during or subsequent to the outbreak of pneumonia among monkeys of Lot 2, monkeys of these two lots being

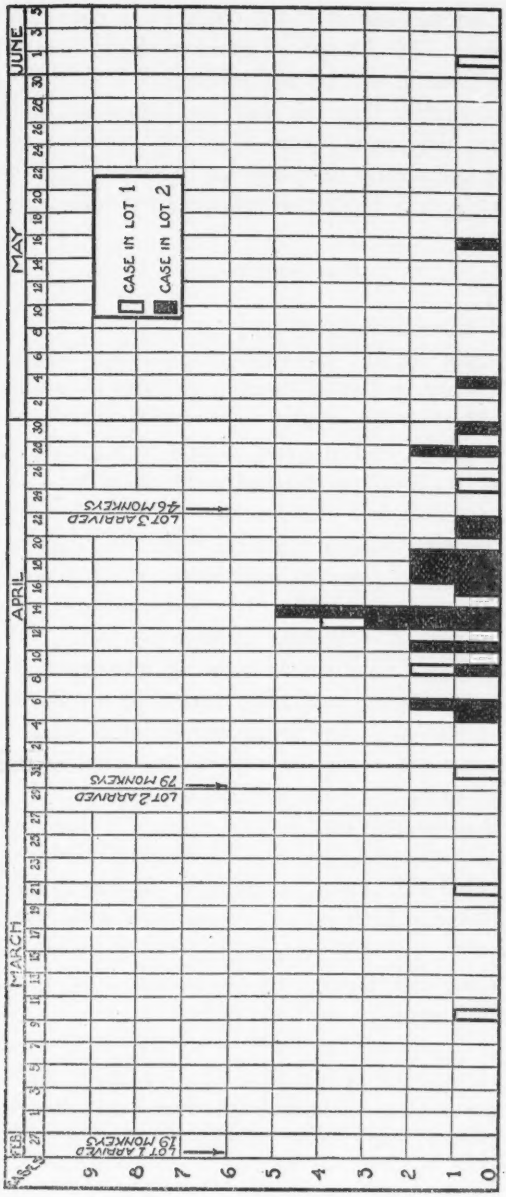
TABLE I.

Spontaneous Pneumonia among Three Lots of Monkeys.

Lot No.	Date of arrival.	No. of monkeys.	No. of cases of pneumonia.	Remarks.
	1919			
1	Feb. 26	19	8	Five of these cases occurred during or after the outbreak of pneumonia among monkeys of Lot 2.
2	Mar. 30	79	28	Overcrowded.
3	Apr. 23	46	0	Kept separate from preceding lots.

in intimate contact with one another. The absence of pneumonia among the monkeys of Lot 3 undoubtedly may be attributed to the fact that they were not allowed to come into contact with the other monkeys and were kept in pairs in separate cages. That a considerable number of unrecorded cases of mild pneumonia with recovery occurred among the monkeys of Lot 2 was subsequently shown by the finding of fibrous pleural adhesions in many monkeys of this lot used later for experimental purposes.

The apparently epidemic character of the spontaneous pneumonia and its relation to the time of arrival of the different lots of monkeys is shown in Text-fig. 1. It will be seen at once that the larger part of the pneumonia occurred shortly after the arrival of the second lot, the curve of incidence being characteristic of an epidemic outbreak



TEXT-FIG. 1. Spontaneous pneumonia among monkeys from February 26 to June 5, 1919.

of disease. The original source of the outbreak of pneumonia could not be determined. That its spread was due chiefly to contact infection is clearly brought out by bacteriological evidence presented below. It is noteworthy that this was the largest lot of monkeys received and that they were under distinctly crowded conditions during the first 3 weeks after their arrival. There seems little doubt that overcrowding greatly facilitated the spread of infection.

Bacteriology.

Bacteriological examination was made in 36 cases—in 2 cases by blood cultures during life, in 2 cases by blood cultures during life and by cultures at autopsy, and in 32 cases by cultures at autopsy alone (Table II). In 32 cases the results were entirely satisfactory, in 2 no growth was obtained, and in 2 the cultures were grossly contaminated by postmortem invaders and had to be discarded. Of the 32 cases in which bacteriological examinations were satisfactory, Pneumococcus Type IV was found in cultures from the lungs or heart's blood, or both, in 28; *Streptococcus hæmolyticus* was found in 2; and a non-hemolyzing streptococcus of the *viridans* group in pure culture in 2 and with *Streptococcus hæmolyticus* in 1. Of course, it remains problematical whether the cases of pneumonia in which streptococci were found were primarily caused by these organisms, or whether the streptococci were secondary invaders which had replaced the pneumococcus at the time of death. Histological study, however, of the 4 cases in which streptococci were found indicated that the lung lesions were at least in part caused by these organisms. Of the 24 cases of fatal pneumococcus pneumonia in which cultures of the heart's blood were made at autopsy, all but 2 showed the pneumococcus present. In the 2 cases associated with *Streptococcus hæmolyticus*, this organism was present in the heart's blood in both, while in the 2 cases associated with *Streptococcus viridans*, cultures of the heart's blood remained sterile in both.

Because of the apparently epidemic character of a considerable part of the spontaneous pneumonia that occurred among the monkeys, it seemed of importance to determine whether or not the strains of Pneumococcus Type IV were immunologically homologous. Accord-

TABLE II.

Bacteriology and Pathology of Spontaneous Pneumonia in Monkeys.

Monkey No.	Date.	Bacteriology.	Anatomical diagnosis.
	1919		
11	Mar. 10	H. B., Pn. IV; L. L., no growth; pericard., Pn. IV.	Lobar pneumonia; L. L., resolving; fibrous pleuritis, left; acute fibrinopurulent pericarditis; acute suppurative meningitis; hypertrophy and dilatation of heart.
12	" 21	H. B., Pn. IV; R. L., Pn. IV.	Lobar pneumonia; R. U., R. M., R. L., engorgement and red hepatization.
28	" 31	H. B., Pn. IV; L. M., Pn. IV; R. L., Pn. IV.	Lobar pneumonia; R. M., R. L., L. M., L. L., red and gray hepatization; acute fibrinous pleuritis, bilateral.
36	Apr. 5	H. B., *S. hæmolyticus*; R. L., *S. hæmolyticus* and *S. viridans*.	Confluent lobular pneumonia, R. L.; acute fibrinous pleuritis, interlobar, right.
37	" 6	H. B., Pn. IV.	Lobar pneumonia; L. U., L. M., R. M., R. L., gray hepatization; acute fibrinous pleuritis, bilateral.
38	" 6	Contaminated.	Lobar pneumonia; R. M., red hepatization.
15	" 9	L. L., Pn. IV.	Lobar pneumonia; L. L., gray hepatization; acute fibrinous pleuritis, left.
35	" 9	Br., Pn. IV; R. L., Pn. IV.	Lobar pneumonia; R. U., R. M., R. L., gray hepatization; acute fibrinous pleuritis, right.
43	" 11	H. B., Pn. IV; lung, contaminated.	Lobar pneumonia; L. M., L. L., gray hepatization; R. M., red hepatization; acute fibrinous pleuritis, bilateral.
44	" 11	H. B., Pn. IV; L. L., Pn. IV.	Lobar pneumonia; L. L., red hepatization; acute fibrinous pleuritis, left.
52	" 13	H. B., *S. hæmolyticus*; L. L., no growth.	Lobar pneumonia; L. L., L. M., L. U., gray hepatization and beginning resolution; acute fibrinous pleuritis, left.
53	" 13	H. B., no growth; L. M., Pn. IV; C., Pn. IV.; Br., Pn. IV.	Lobar pneumonia; R. M., gray hepatization; C., L. M., red hepatization; R. U., engorgement; acute fibrinous pleuritis, right.
54	" 13	H. B., Pn. IV; R. L., Pn. IV.	Lobar pneumonia; R. L., gray hepatization; acute fibrinous pleuritis, right.

H. B. indicates heart's blood; B. C., blood culture; R. L., C., L. L., etc., lobes of the lung; Pl., pleura; Br., bronchus.

TABLE II—*Continued.*

Monkey No.	Date.	Bacteriology.	Anatomical diagnosis.
	1919		
18	Apr. 13	H. B., no growth; R. L., *S. viridans*; L. M., *S. viridans*.	Interstitial and lobular pneumonia, R. M., R. L., L. L.; acute fibrinous pleuritis, bilateral.
55	" 14	H. B., Pn. IV; L. L., Pn. IV.	Lobar pneumonia; R. M., R. L., L. M., L. L., red and gray hepatization; acute fibrinous pleuritis, bilateral.
56	" 14	H. B., Pn. IV; L. L., Pn. IV.	Lobar pneumonia; L. L., gray hepatization; acute fibrinous pleuritis, left.
57	" 14	H. B., no growth; L. L., no growth.	Lobar pneumonia; L. L., gray hepatization; acute fibrinous pleuritis, left.
58	" 14	Contaminated.	Lobar pneumonia; R. M., R. L., gray hepatization; acute fibrinous pleuritis, right.
59	" 14	H. B., Pn. IV; L. U., no growth.	Lobar pneumonia; L. U., L. M., gray hepatization; R. M., resolution; acute fibrinous pleuritis, bilateral.
34	" 16	H. B., Pn. IV; L. L., no growth; R. L., contaminated.	Lobar pneumonia; L. U., L. M., engorgement; L. L., gray hepatization; R. L., resolution; acute fibrinous pleuritis, bilateral.
60	" 17	H. B., no growth; L. L., *S. viridans*.	Interstitial and lobar pneumonia; R. U., R. M., R. L., gray hepatization; L. L., interstitial and lobular pneumonia; acute fibrinous pleuritis, bilateral.
61	" 17	H. B., Pn. IV; L. L., Pn. IV.	Lobar pneumonia; L. U., L. M., L. L., red and gray hepatization; acute fibrinous pleuritis, left.
62	" 18	H. B., Pn. IV; L. L., no growth.	Lobar pneumonia; R. M., C., red hepatization; L. L., resolution; acute fibrinous pleuritis, left.
50	" 18	H. B., Pn. IV; L. M., Pn. IV; L. L., Pn. IV.	Lobar pneumonia; L. M., L. L., gray hepatization; L. U., C., red hepatization; acute fibrinous pleuritis, left.
68	" 19	H. B., Pn. IV; R. L., contaminated.	Lobar pneumonia; R. M., R. L., L. M., red hepatization; acute fibrinous pleuritis, right.
69	" 19	H. B., Pn. IV; L. U., Pn. IV.	Lobar pneumonia; L. U., L. M., gray hepatization; acute fibrinous pleuritis, left.
70	" 21	H. B., Pn. IV; R. M., Pn. IV.	Lobar pneumonia; L. U., L. M., gray hepatization; R. M., R. L., red hepatization; acute fibrinous pleuritis, bilateral.

TABLE II—*Concluded.*

Monkey No.	Date.	Bacteriology.	Anatomical diagnosis.
	1919		
74	Apr. 22	H. B., Pn. IV; L. L., no growth.	Lobar pneumonia; L. L., resolution; R. M., R. L., red hepatization; acute fibrinous pleuritis, bilateral.
13	" 25	H. B., Pn. IV; R. L., no growth; pericard., Pn. IV.	Lobar pneumonia; R. M., R. L., organization; L. M., L. L., resolution and organization; acute fibrinous pleuritis, bilateral; acute serofibrinous pericarditis; hypertrophy and dilatation of heart.
71	" 28	H. B., no growth; L. U., Pn. IV.	Lobar pneumonia; L. U., gray hepatization; acute fibrinous pleuritis, left.
73	" 28	H. B., Pn. IV; L. L., no growth.	Lobar pneumonia; L. M., L. L., C., R. M., R. L., gray hepatization; acute fibrinous pleuritis, bilateral.
21	" 29	L. M., no growth; pericard., no growth.	Lobar pneumonia; L. U., L. M., resolution; acute fibrinous pleuritis, left; acute fibrinous pericarditis.
47	" 30	B. C., Pn. IV.	Lobar pneumonia; L. M., L. L., gray hepatization and resolution; R. M., resolution; acute fibrinous pleuritis, left.
84	May 4	H. B., Pn. IV; R. L., contaminated	Lobar pneumonia; L. L., resolution; R. L., C., gray hepatization; acute fibrinous pleuritis, bilateral.
97	" 16	H. B., Pn. IV; L. L., Pn. IV; Pl., Pn. IV; pericard., Pn. IV; Br., Pn. IV.	Lobar pneumonia; R. U., R. M., R. L., C., L. M., L. L., red and gray hepatization; acute fibrinopurulent pleuritis, bilateral; acute fibrinopurulent pericarditis; acute fibrinous peritonitis, localized.
19	June 1	B. C., Pn. IV.	(Clinical diagnosis, lobar pneumonia. Recovered; no autopsy.)

ingly, three rabbits were immunized against the strains isolated from Monkeys 19, 53, and 97, respectively, and the immune sera so prepared were used for cross-agglutination tests. Of the twenty-eight strains isolated, twenty-one had been preserved and were available for agglutination. The results are shown in Table III.

It will be seen from Table III that nine strains (from Monkeys 13, 19, 34, 35, 47, 73, 74, 84, and 97) fell into one immunologically homologous group, that five strains (from Monkeys 50, 53, 61, 69, and 70) fell

into another homologous group, and that there remained but seven strains which fell into neither of these groups. Though some of the strains remain unclassified, the evidence obtained is amply sufficient to demonstrate that the outbreak of spontaneous pneumonia was associated with the spread of infection from monkey to monkey. This becomes all the more apparent when the immunological groups are cor-

TABLE III.

Cross-Agglutination Reactions of Strains of Pneumococcus Type IV Isolated from Monkeys with Spontaneous Lobar Pneumonia.

Strain No.	Immune sera.			Normal serum.
	No. 19.	No. 53.	No. 97.	
12	−	−	−	−
13	++	−	++	−
15	−	−	−	−
19	++	−	++	−
28	−	−	−	−
34	++	−	++	−
35	++	−	++	−
44	−	−	−	−
47	++	−	++	−
50	−	++	−	−
53	−	++	−	−
55	−	−	−	−
56	−	−	−	−
61	−	++	−	−
69	−	++	−	−
70	−	++	−	−
71	−	−	−	−
73	++	−	++	−
74	++	−	++	−
84	++	−	++	−
97	++	−	++	−

related with the dates of death from pneumonia in the individual cases. The strains isolated from Monkeys 12 and 28 did not fall into either of the established groups. Both these monkeys were from Lot 1 and developed pneumonia before the arrival of Lot 2, dying on March 21 and 31, respectively. Monkeys 53, 61, 50, 69, and 70, whose pneumonia was due to the same strain of Pneumococcus Type IV, died on April 13, 17, 18, 19, and 21, respectively, within a period of 9 days.

Of the nine cases of pneumonia due to the other single strain of Pneumococcus Type IV, Monkeys 35, 34, 74, 13, 73, 84, and 97 died on April 9, 16, 22, 25, 28, May 4, and 16, respectively. Monkey 47 developed pneumonia on April 30 and recovered by crisis on May 9. These eight cases were sufficiently near to each other to have contracted pneumonia by contact infection from a preceding case. The ninth case caused by a pneumococcus of this type, Monkey 19, developed pneumonia on June 1, and had, therefore, no direct relation with the epidemic. Monkey 19 was at the time, however, in a cage adjoining that of Monkey 111. At this time Monkey 111 was in the active stage of an experimental pneumonia produced by inoculation with the strain of Pneumococcus Type IV which had been isolated at necropsy from Monkey 97 of this group. It is presumable that Monkey 19 acquired its spontaneous infection from this source.

Clinical Features.

Clinical observations upon spontaneous lobar pneumonia were made in two instances. In a third case the course of pneumococcus pericarditis following spontaneous pneumonia was studied. The protocols with accompanying clinical charts follow.

Protocol 1.—Monkey 47. *Macacus syrichtus*, male; weight 2,710 gm. Apr. 29, 1919. Well and active. Apr. 30, a.m. Well and active. p.m. Appears quiet. May 1. Appears sick. Respirations moderately accelerated. Temperature elevated. No abnormal signs in chest. May 2. Very sick; sitting huddled up in corner of cage, breathing rapidly with expiratory grunt; blood culture shows Pneumococcus Type IV. May 3. Condition the same; moderate dullness and suppressed breathing over left lower lobe; distant bronchial breathing heard in left axilla. May 4. Appears better; respirations rapid and labored. May 5 to 8. Condition the same; coughs occasionally; breathing rapidly. May 9. Appears well and active; temperature fallen to normal by crisis. May 10. Well and active. Killed.

The temperature, leucocyte curve, and results of blood cultures are shown in Text-fig. 2.

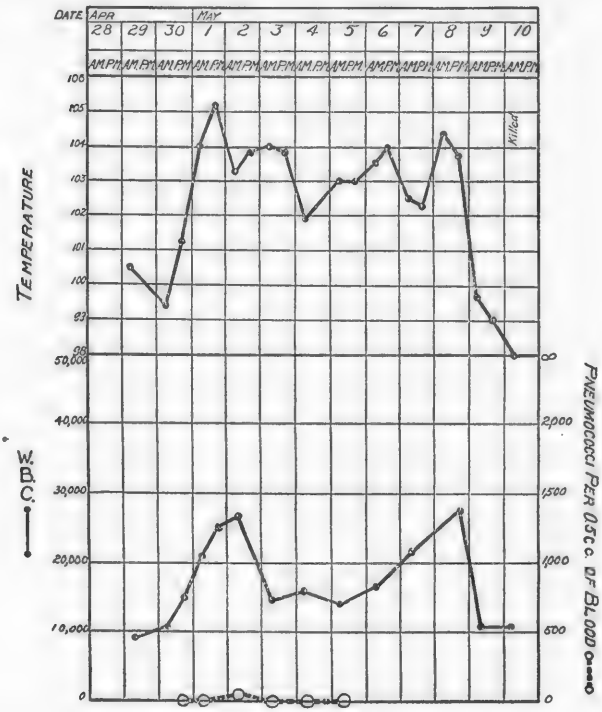
Autopsy. Anatomical Diagnosis.—Lobar pneumonia; left middle and lower lobes, gray hepatization and beginning resolution; right middle lobe resolving; acute fibrinous pleuritis, left.

Cultures.—Heart's blood, no growth; left lower lobe, no growth.

Protocol 2.—Monkey 19. *Macacus syrichtus*, male; weight 4,000 gm. May 29 to 31, 1919. Appears well and active. June 1. Quiet; temperature elevated; breathing rapidly; leucocytosis. June 2. Appears sick; refuses food; respirations rapid and labored; blood culture shows Pneumococcus Type IV. June 3 to 5. Condition the same. June 6. Appears better; still breathing rapidly. June 7. Better; temperature falling. June 8. Appears well and active; temperature normal. Remained well. No autopsy.

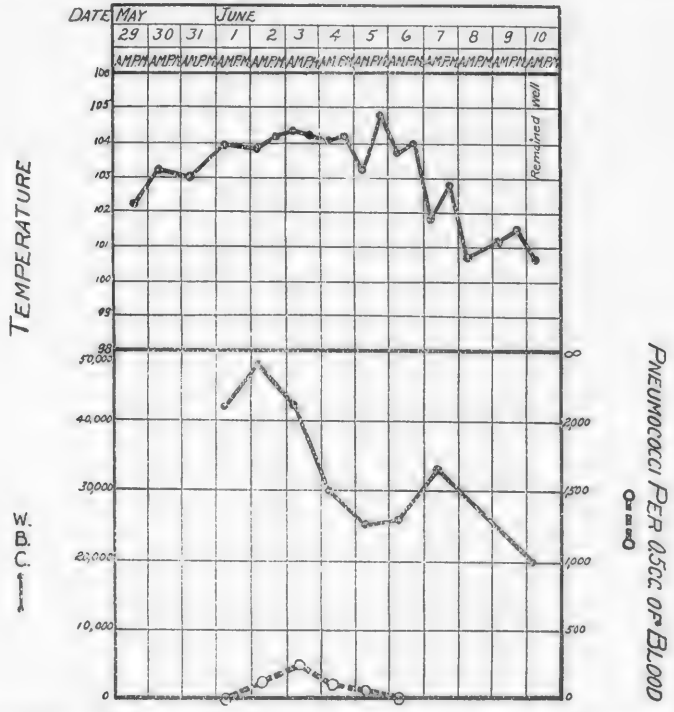
The temperature, leucocyte curve, and results of blood cultures are shown in Text-fig. 3.

Both monkeys presented the typical picture and ran the characteristic course of lobar pneumonia, identical with the disease experi-



TEXT-FIG. 2. Monkey 47. Spontaneous lobar pneumonia, Pneumococcus Type IV.

mentally produced by the intratracheal injection of pneumococcus, and like it, corresponding in all respects to lobar pneumonia in man. The leucocyte reaction, characteristic of relatively mild cases that recover, is well shown, as is the temporary invasion of the blood by pneumococci and recovery by crisis.

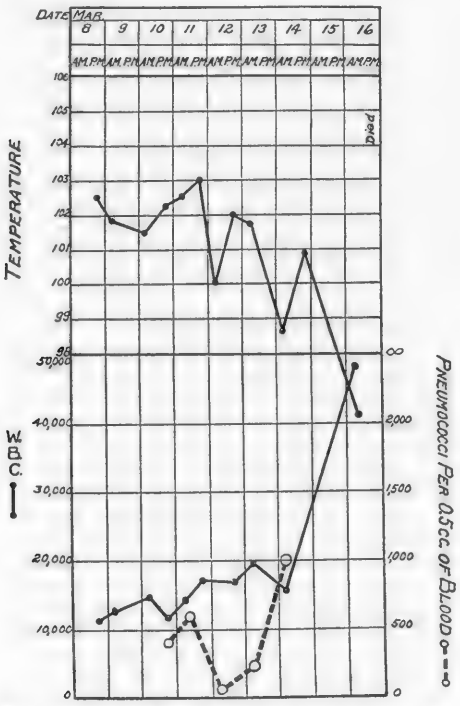


TEXT-FIG. 3. Monkey 19. Spontaneous lobar pneumonia, Pneumococcus Type IV.

Protocol 3.—Monkey 11. *Macacus syrichtus*, male; weight 4,325 gm. Mar. 8, 1919. Appears moderately sick; breathing slightly accelerated; lungs clear. X-ray of chest shows cardiac shadow moderately enlarged and distinctly globular; lungs show no evidence of consolidation (Fig. 1). Mar. 10. Condition the same. Blood culture shows Pneumococcus Type IV. Mar. 11 to 13. Condition the same. Lungs clear. Mar. 14. Right leg appears paralyzed. Mar. 16. Very sick; lying down; semicomatose. 9.30 p.m. Died.

The temperature, leucocyte curve, and results of blood cultures are shown in Text-fig. 4.

Autopsy. Anatomical Diagnosis.—Resolving lobar pneumonia, left lower lobe; acute fibrinopurulent pericarditis; acute suppurative meningitis; hypertrophy and dilatation of the heart; fibrous pleuritis, left.



TEXT-FIG. 4. Monkey 11. Fibrinopurulent pericarditis with terminal meningitis following spontaneous lobar pneumonia, Pneumococcus Type IV.

Pericardium.—Appears considerably thickened, and on opening the sac a considerable quantity of turbid fluid is found. The parietal and visceral layers of the pericardium are everywhere covered with a shaggy coat of fibrin which is easily peeled off from the underlying membrane. *Heart.*—Moderate hypertrophy and dilatation. *Left pleural cavity.*—Contains many fibrous adhesions between the parietal and visceral pleuræ. *Lungs.*—Crepitant and pale pink, except the left lower lobe which is a translucent gray and doughy. *Brain.*—Appears normal.

Cultures.—Heart's blood, Pneumococcus Type IV; pericardial exudate, Pneumococcus Type IV; left lower lobe, no growth.

Microscopic Examination.—Heart.—Myocardium shows cloudy swelling; on the epicardium there is a dense layer of fibrin everywhere infiltrated with polymorphonuclear leucocytes and lymphoid cells. *Left lower lobe.*—Shows the last stages of a resolving pneumonia. *Medulla.*—The meninges are densely infiltrated with polymorphonuclear leucocytes, which form a thick layer over the surface.

There seems little question that the pneumococcus pericarditis in Monkey 11 developed following spontaneous lobar pneumonia. It is presumable that the terminal meningitis was also caused by Pneumococcus Type IV, although bacteriological evidence is lacking. Both conditions were similar to pneumococcus pericarditis and meningitis as seen in man complicating lobar pneumonia.

Pathology.

Thirty-two cases presented at autopsy the characteristic pathology of pneumococcus lobar pneumonia (Table II), resembling in all respects the experimental disease produced by the intratracheal injection of pneumococci as described in a preceding paper.[2] Pneumococcus pericarditis occurred in four cases, empyema in one, and meningitis in one. Acute fibrinous pleuritis was present in all but two cases. In the majority of cases the pneumonia was extensive, more than one lobe being involved in twenty-four cases; seventeen cases showed bilateral pneumonia. A characteristic feature of these cases was the presence of an advanced stage of gray hepatization or beginning resolution in some lobes, while in others the picture of engorgement and red hepatization was found, indicating that a spread of the process had occurred during the course of the disease. In eight cases only one lobe was involved, in one of which death was due to a complicating pericarditis. The right and left sides were about equally involved, the lower and middle lobes nearly four times as frequently as the upper lobes. As in experimental pneumonia in monkeys partial organization occurred somewhat more frequently than it is believed to occur in lobar pneumonia in man. Macroscopically the cut surfaces of the lungs presented the characteristic appearances of the stages of engorgement, red and gray hepatization, and beginning resolution. The lobar character of the consolidation

is well shown in photographs of the lungs from Monkeys 43 and 57 (Figs. 2 and 3) and of a stained histological section of the right lower lobe from Monkey 84 (Fig. 4). Location of the consolidation in the proximal portion of the lobe when complete lobar consolidation did not develop is shown in Fig. 5. Cases associated with streptococci are too few in number to warrant description. The character of the lesion in Monkey 36 is shown in Fig. 6.

In a similar manner study of histological sections (Figs. 7 to 16) has shown the spontaneous disease to be identical with that experimentally produced, leaving no doubt that the pathogenesis of the two is the same. A few illustrative protocols are given.

Protocol 4.—Monkey 35. *Macacus syrichtus*, female. Autopsy performed Apr. 9, 1919, 8 hours post mortem.

Anatomical Diagnosis.—Lobar pneumonia; right upper, middle, and lower lobes, stage of gray hepatization; acute fibrinous pleuritis, right; cloudy swelling of viscera.

Pericardial sac.—Contains no fluid and appears normal. *Heart.*—Of normal size; myocardium firm; valves normal. *Pleural cavities.*—Contain no fluid. There is a fresh deposit of fibrin on the visceral and parietal layers of the right pleura. The left pleura is smooth and glistening. *Lungs.*—The right upper, middle, and lower lobes are completely consolidated, except for a small portion along the anterior margins; surfaces dark red and roughened with fibrin. The lobes are plastered together with a fibrinous exudate. Cut surface of the right lung is reddish gray and finely granular. Left lung is pale pink, somewhat collapsed, and crepitant throughout. The trachea and bronchi are filled with rusty mucoid material. *Other organs.*—Abdominal organs normally disposed; liver and kidneys show cloudy swelling.

Cultures.—Right lower lobe, Pneumococcus Type IV; right bronchus, Pneumococcus Type IV.

Microscopic Examination.—Right lower lobe.—Except at the periphery the alveoli are filled with a dense exudate of polymorphonuclear leucocytes, the structure of the alveolar walls being well preserved (Fig. 7). The perivascular tissue about the larger vessels is edematous and moderately infiltrated with polymorphonuclear leucocytes, large mononuclear cells, and lymphocytes, the lymphatics being distended with similar cells (Fig. 8). The bronchi and bronchioles are filled with exudate. At the periphery there is marked congestion of the smaller vessels and alveolar capillaries and perivascular edema. The alveoli contain a serous exudate with few cells (Fig. 9). There is a dense exudate of polymorphonuclear leucocytes and fibrin on the pleural surface. There are many pneumococci in all parts of the section, both in the alveoli and in the alveolar walls at the periphery.

Protocol 5.—Monkey 53. *Macacus syrichtus*, male. Autopsy performed Apr. 13, 1919.

Anatomical Diagnosis.—Lobar pneumonia; right middle lobe, gray hepatization; cardiac lobe and left middle lobe, red hepatization; right upper lobe, engorgement and edema; acute fibrinous pleuritis, right.

Pericardial sac.—Contains no fluid and appears normal. *Heart.*—Markedly enlarged, both ventricles being dilated; myocardium and valves appear normal. *Pleural cavities.*—Contain no fluid. There is a deposit of fibrin on the pleural surfaces in the lower part of the right pleural cavity. The left pleura is smooth and glistening. *Lungs.*—The right middle lobe is firmly consolidated throughout, the cut surface being yellowish gray and finely granular. The right upper lobe shows a small patch of beginning consolidation near the hilum, deep red in color, the cut surface oozing abundant frothy fluid. The cardiac lobe is moderately firm throughout, deep red in color, the cut surface moist. The left middle lobe shows a similar picture. The right lower, left upper, and left lower lobes are pale pink and crepitant throughout. *Abdominal organs.*—Show nothing of interest.

Cultures.—Heart's blood, no growth; left middle lobe, Pneumococcus Type IV; cardiac lobe, Pneumococcus Type IV; bronchus, Pneumococcus Type IV.

Microscopic Examination.—Right middle lobe.—The alveoli are everywhere filled with a dense exudate of polymorphonuclear leucocytes; in some places these show marked disintegration and there is beginning desquamation of alveolar epithelium. There is moderate perivascular edema and infiltration with polymorphonuclear leucocytes about some of the smaller vessels. Many pneumococci are present in the pneumonic exudate. *Right upper lobe.*—Central portion shows marked engorgement of vessels and alveolar capillaries, serous exudate with fibrin network in the alveoli, and some exudation of red blood corpuscles; tissue appears normal at the periphery. Pneumococci are numerous in the walls of the alveoli and in the alveolar exudate. They are also frequently seen in the apparently uninvolved peripheral portions of the lobe where they are confined within the walls of the alveoli.

Protocol 6.—Monkey 97. *Macacus syrichtus*, female. Autopsy performed May 16, 1919, immediately after death.

Anatomical Diagnosis.—Lobar pneumonia; right lower, middle, cardiac, and upper lobes, left lower and middle lobes, red and gray hepatization; acute fibrinopurulent pericarditis; acute fibrinopurulent pleuritis, bilateral; acute fibrinous peritonitis, localized; cloudy swelling of viscera.

Pericardial sac.—Contains about 10 cc. of yellowish, turbid fluid, in which are flakes of fibrin. Visceral and parietal layers of the pericardium are covered with a shaggy fibrinous exudate. *Heart.*—Of normal size; myocardium and valves appear normal. *Pleural cavities.*—Both contain about 25 cc. of turbid, yellowish fluid. There is abundant fibrinous exudate over both lower and middle lobes. *Lungs.*—The right middle and lower lobes are firmly consolidated throughout, the cut surfaces being reddish gray in the center, deep red and very moist at

the periphery. The upper half of the cardiac lobe and a small portion of the right upper lobe near the hilum present a similar appearance. The left middle lobe and the upper and posterior half of the left lower lobe are firmly consolidated, the cut surfaces reddish gray and finely granular. The remainder of the lungs is pinkish and air-containing, but the cut surfaces yield a large amount of frothy fluid. Trachea and bronchi are filled with bloody mucus. *Other organs.*—There are a few fresh fibrinous adhesions between the diaphragm and the upper surface of the liver. Otherwise abdominal organs appear normal, except for cloudy swelling of liver and kidneys.

Cultures.—Heart's blood, Pneumococcus Type IV; pericardial fluid, Pneumococcus Type IV; right pleural fluid, Pneumococcus Type IV; left lower lobe, Pneumococcus Type IV; bronchus, Pneumococcus Type IV.

Microscopic Examination.—Left lower lobe.—Section shows typical picture of gray hepatization, the alveoli being filled with a dense exudate of polymorphonuclear leucocytes. The structure of the alveolar walls is well preserved. The peribronchial and perivascular tissue is edematous and infiltrated with leucocytes and mononuclear cells (Fig. 11). At the periphery there is a zone of congestion and edema, the alveoli containing only a serous exudate. In places the alveoli are filled with red blood corpuscles. The right lower lobe presents a similar picture. Pneumococci are plentiful in the exudate and in the edematous alveoli. They are also found in considerable numbers in the alveolar walls in uninvolved portions of the lung near the periphery. In such areas they are not found in the alveolar lumen. *Left pleura.*—Thick exudate of fibrin and leucocytes (Fig. 12).

Protocol 7.—Monkey 13. *Macacus syrichtus*, male. Autopsy performed Apr. 25, 1919, 12 hours post mortem.

Anatomical Diagnosis.—Lobar pneumonia; right middle and lower lobes, organization; left middle and lower lobes, resolution and organization; acute serofibrinous pericarditis; acute fibrinous pleuritis, bilateral; hypertrophy and dilatation of heart.

Pericardial sac.—Contains a slightly increased amount of cloudy fluid. *Heart.*—Greatly enlarged, the right auricle and ventricle being markedly dilated; myocardium and valves show no abnormalities. *Pleural cavities.*—Contain no fluid. There are firm fibrinous adhesions in both pleural cavities which show beginning organization. *Lungs.*—Entire left lung is flabby and has a rubbery consistency. Upper and middle lobes are firmly bound together; cut surface is pinkish, moist, and for the most part air-containing. The right middle and lower lobes are firmly consolidated, presenting on section a uniform, grayish, translucent surface. *Abdominal organs.*—Show cloudy swelling.

Cultures.—Heart's blood, Pneumococcus Type IV; pericardial fluid, Pneumococcus Type IV; right lower lobe, no growth.

Microscopic Examination.—Right lower lobe.—Section shows advanced organization. There is almost complete absence of exudate in the alveoli, and the latter have been greatly compressed and distorted. Some alveoli show marked

dilatation. In many places the alveolar walls are greatly thickened and the alveoli are filled with plugs of new formed connective tissue which is vascular and is infiltrated with lymphocytes (Fig. 14). There is marked perivascular organization with infiltration of lymphocytes and plasma cells. Similar changes are noted around the bronchi, though less marked. No bacteria are seen. *Left lower lobe.*—Presents a similar picture.

The foregoing protocols clearly show that the pathology of spontaneous lobar pneumonia in monkeys is identical with that of lobar pneumonia experimentally produced. Since monkeys which died of spontaneous pneumonia had reached a well advanced stage of the disease, the purely interstitial character of the stage of invasion, as described in connection with the pathogenesis of experimental pneumonia, was not encountered. The frequent occurrence, however, even in the later stages of varying degrees of perivascular edema and infiltration, the presence of pneumococci in the alveolar walls of uninvolved peripheral portions of the lung, and the more advanced character of the pneumonic process in the areas about the larger vessels and bronchi can leave little doubt that the mode of involvement of the lung was the same as that which occurs when pneumococci are experimentally injected into the trachea.

SUMMARY.

Spontaneous pneumonia occurred to a considerable extent among stock monkeys at the Army Medical School. This pneumonia occurred chiefly in the form of an epidemic outbreak shortly after the arrival of a large shipment of monkeys, and was shown to be due in large part to transmission of infection from monkey to monkey, either directly or indirectly. That spread of the epidemic was facilitated by overcrowding was indicated by the fact that in a subsequent shipment of monkeys, which were kept in pairs in separate cages and were not allowed to come into contact with the monkeys among which the epidemic occurred, no cases of spontaneous pneumonia developed.

The close analogy between the epidemic of lobar pneumonia that occurred among the monkeys and similar epidemics of lobar pneumonia that occurred during the war among certain groups of newly drafted troops shortly after their arrival at camp is very striking,

and would seem to indicate that pneumococcus pneumonia may become an epidemic disease among groups of susceptible individuals when they are assembled under conditions that facilitate the ready transfer of infection from individual to individual.

Bacteriological examination showed the spontaneous pneumonia to be due in the great majority of cases to infection with Pneumococcus Type IV. Immunological classification of the strains of pneumococci by cross-agglutination tests showed that the majority fell into two biological groups. Two cases were apparently caused by *Streptococcus hæmolyticus*, two by *Streptococcus viridans*.

The clinical course of spontaneous pneumococcus pneumonia in monkeys was characterized by sudden onset, high sustained temperature, leucocytosis, rapid respiration with expiratory grunt, cough, physical signs of consolidation, invasion of the blood by pneumococci, and termination in death or recovery by crisis about the 7th to 9th day. In a few instances the disease was complicated by acute fibrinopurulent pericarditis, by empyema, and by purulent meningitis. It was, therefore, clinically identical with lobar pneumonia experimentally produced in monkeys and with lobar pneumonia in man.

Study of the pathology of spontaneous pneumococcus pneumonia in monkeys showed that it presented the characteristic picture of lobar pneumonia, both macroscopically and microscopically, and was in all respects comparable with the pathology of lobar pneumonia experimentally produced in monkeys and of lobar pneumonia in man.

CONCLUSIONS.

1. Monkeys in captivity are subject to spontaneous lobar pneumonia.

2. Spontaneous lobar pneumonia in monkeys is caused by the pneumococcus.

3. Lobar pneumonia in monkeys may occur as an epidemic disease and be due to spread of infection from animal to animal when conditions which favor contact infection are present.

4. Spontaneous lobar pneumonia in monkeys is identical in its clinical features, complications, and pathology with lobar pneumonia experimentally produced in monkeys by the intratracheal injection of pneumococcus and with lobar pneumonia in man.

EXPLANATION OF PLATES.

PLATE 53.

FIG. 1. Monkey 11. Fibrinopurulent pericarditis following spontaneous lobar pneumonia, Pneumococcus Type IV. X-ray of chest.

PLATE 54.

FIG. 2. Monkey 43. Spontaneous lobar pneumonia, Pneumococcus Type IV. Cut surface of lungs showing gray hepatization of left lower and middle lobes; red hepatization of right middle lobe.

PLATE 55.

FIG. 3. Monkey 57. Spontaneous lobar pneumonia, Pneumococcus Type IV. Cut surface of lungs showing gray hepatization and beginning resolution of left lower lobe.

PLATE 56.

FIG. 4. Monkey 84. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section completely through right lower lobe showing lobar character of consolidation; stage of gray hepatization.

FIG. 5. Monkey 59. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section completely through right middle lobe showing hepatization of the part of the lobe proximal to the hilum, perivascular edema and infiltration, and peribronchial and interstitial involvement with absence of exudate in the alveoli and bronchioles in the distal portion of the lobe.

PLATE 57.

FIG. 6. Monkey 36. Spontaneous pneumonia, *Streptococcus hæmolyticus*. Section completely through the right lower lobe.

PLATE 58.

FIG. 7. Monkey 35. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from right lower lobe, stage of gray hepatization, showing alveoli filled with leucocytic exudate. × 100.

PLATE 59.

FIG. 8. Monkey 35. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from right lower lobe, stage of gray hepatization, showing perivascular edema and moderate infiltration, perivascular lymphatics filled with leucocytes, and exudate in the lumen of a small bronchus. × 100.

PLATE 60.

FIG. 9. Monkey 35. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from margin of right lower lobe showing vascular and capillary engorgement and serous exudate in alveoli. × 100.

FIG. 10. Monkey 28. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from right lower lobe, stage of red hepatization, showing alveolar exudate of coagulated serum, fibrin, red blood cells, and a few polymorphonuclear leucocytes. × 100.

PLATE 61.

FIG. 11. Monkey 97. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from left lower lobe showing edema and infiltration of bronchial wall. × 100.

PLATE 62.

FIG. 12. Monkey 97. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from left lower lobe showing thick exudate of fibrin and leucocytes on the pleura. × 64.

FIG. 13. Monkey 47. Spontaneous lobar pneumonia, Pneumococcus Type IV. Monkey killed 24 hours after recovery by crisis. Section from left lower lobe (beginning resolution) showing intense leucocytic infiltration of perivascular tissue. × 100.

PLATE 63.

FIG. 14. Monkey 13. Spontaneous lobar pneumonia, death resulting from a complicating pneumococcus pericarditis. Section from right lower lobe showing alveoli filled with plugs of young connective tissue and beginning organization of perivascular tissue. × 100.

PLATE 64.

FIG. 15. Monkey 62. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from cardiac lobe showing early alveolar lesion with marked leucocytic infiltration of alveolar walls and in places beginning exudation into the alveoli, the alveolar duct and atrium being free from exudate. × 83.

FIG. 16. Monkey 70. Spontaneous lobar pneumonia, Pneumococcus Type IV. Section from right lower lobe showing invasion of alveolar walls by pneumococci beyond the margin of the consolidated area. × 1,000.

FIG. 1.

(Blake and Cecil: Experimental pneumonia. III.)

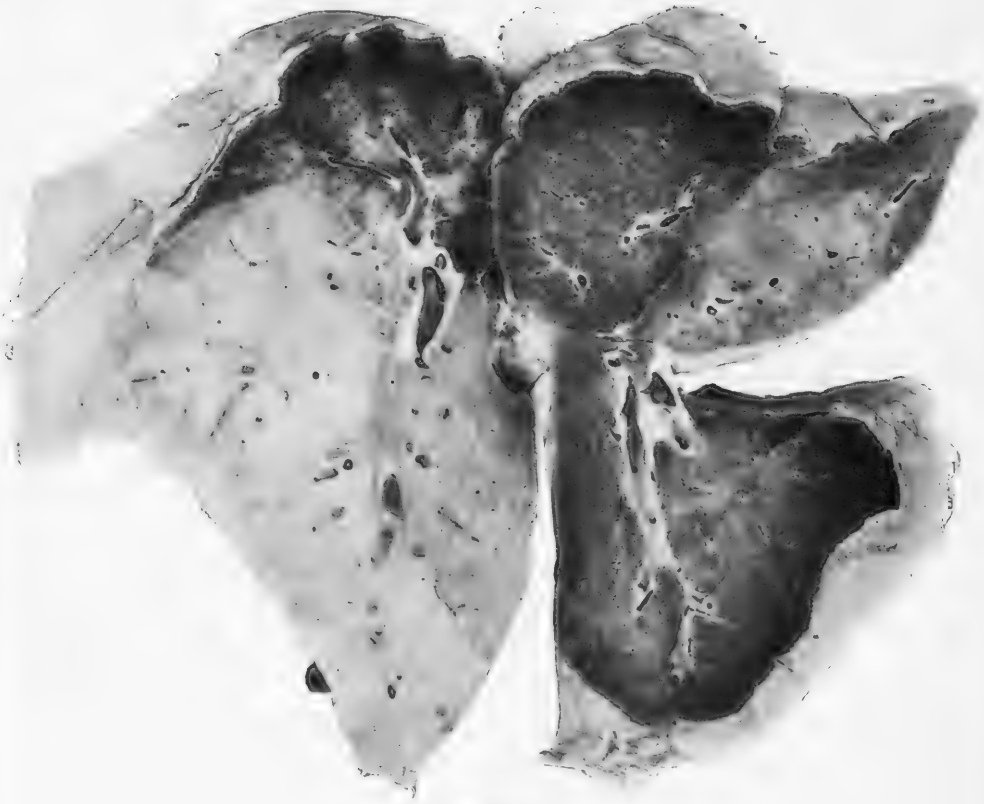


FIG. 2.

(Blake and Cecil: Experimental pneumonia. III.)

FIG. 3.

(Blake and Cecil: Experimental pneumonia. III.)

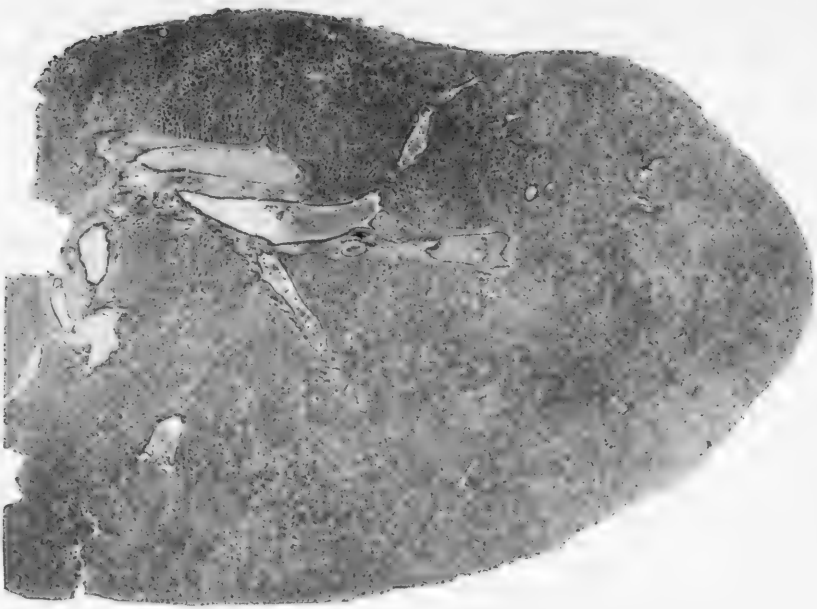


FIG. 4.

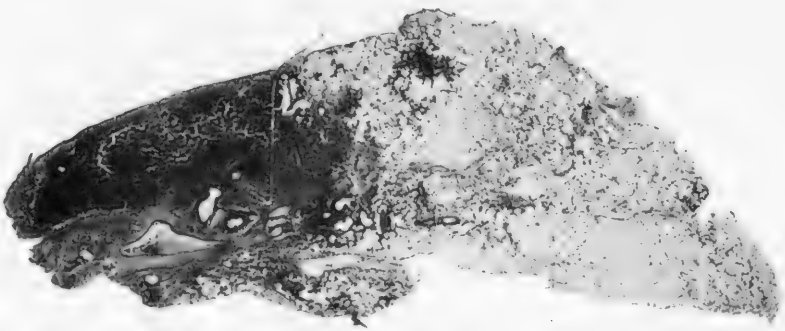


FIG. 5.

(Blake and Cecil: Experimental pneumonia. III.)

FIG. 6.

(Blake and Cecil: Experimental pneumonia. III.)

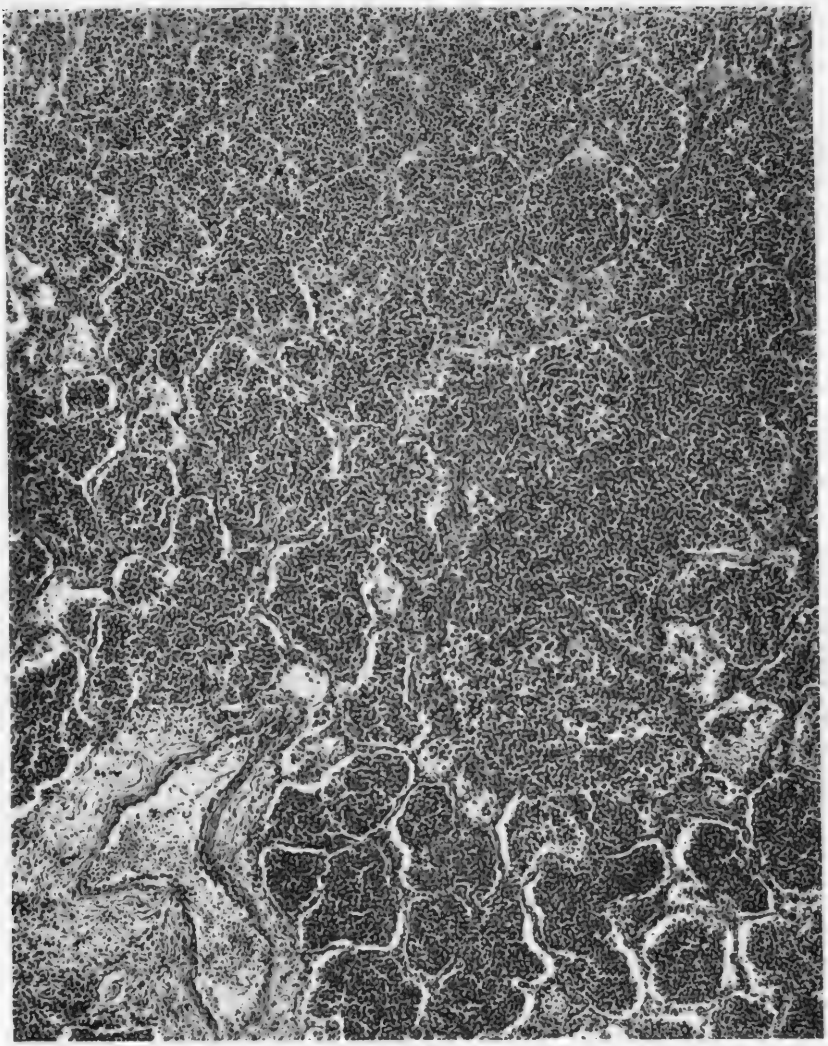


FIG. 7.

(Blake and Cecil: Experimental pneumonia. III.)

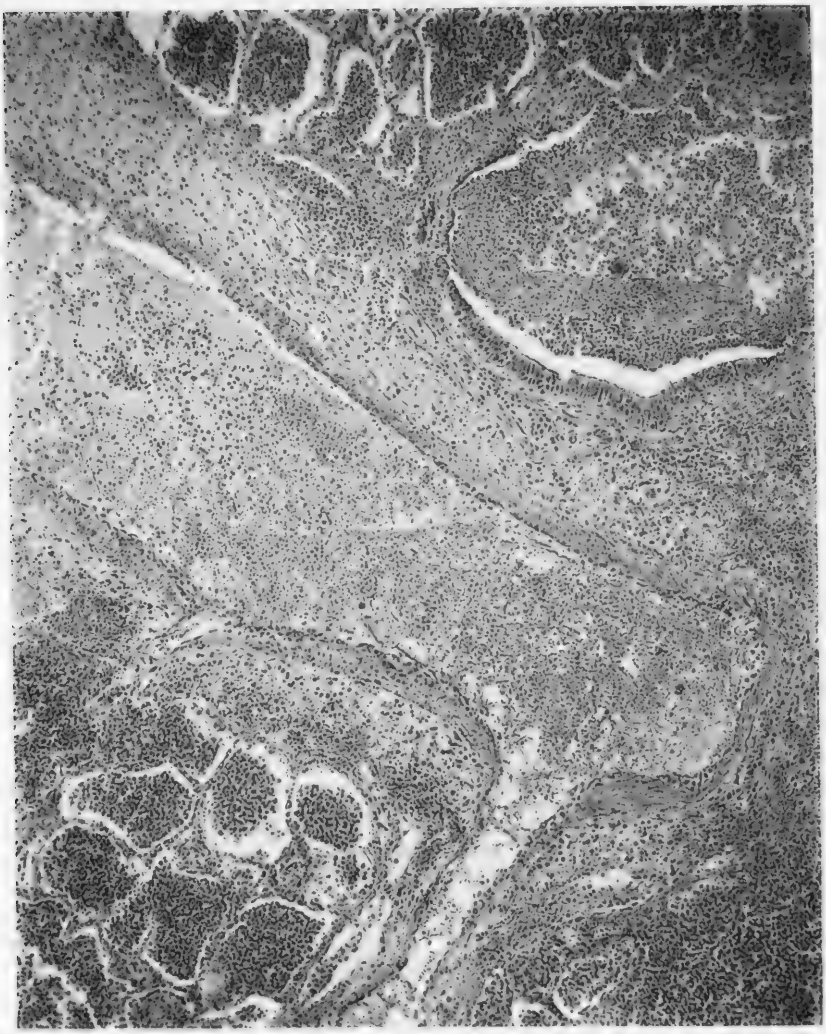


FIG. 8.

(Blake and Cecil: Experimental pneumonia. III.)

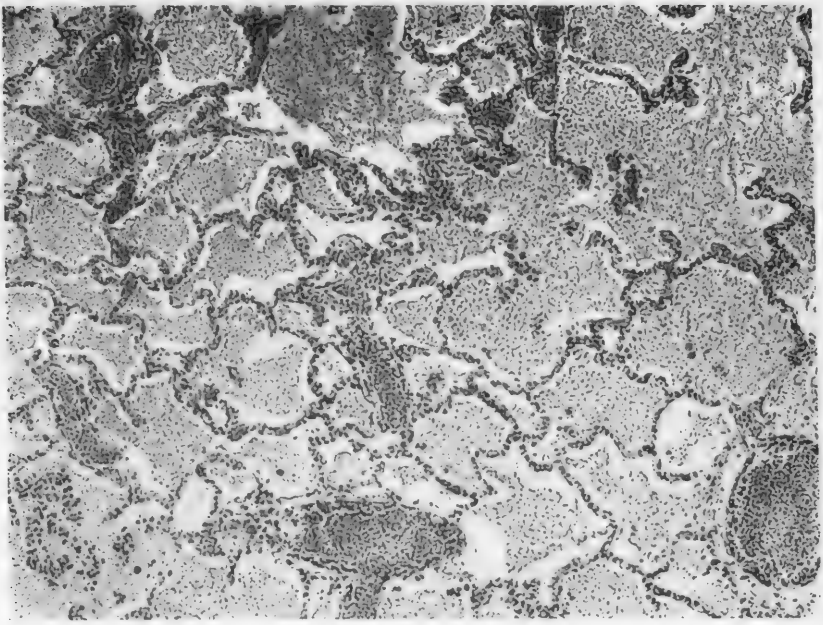


FIG. 9.

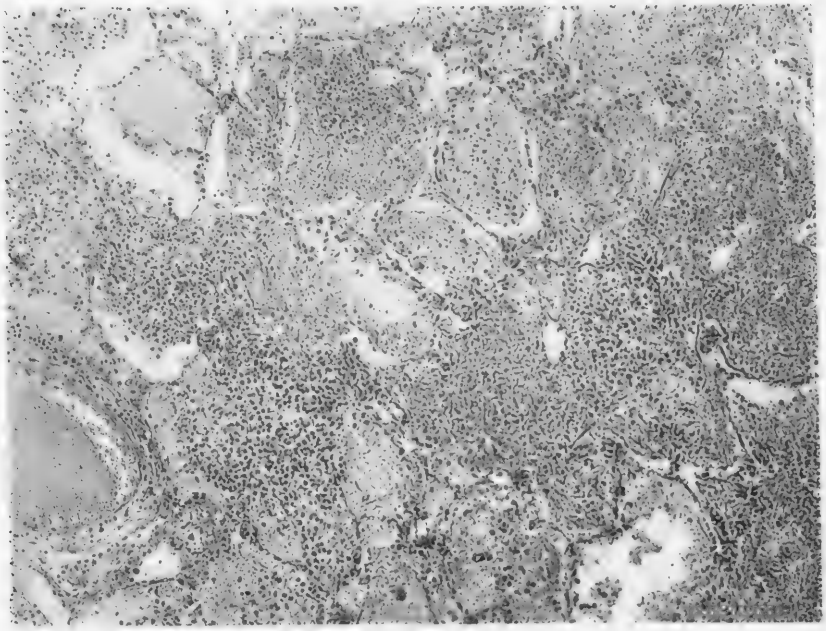


FIG. 10.

(Blake and Cecil: Experimental pneumonia. III.)

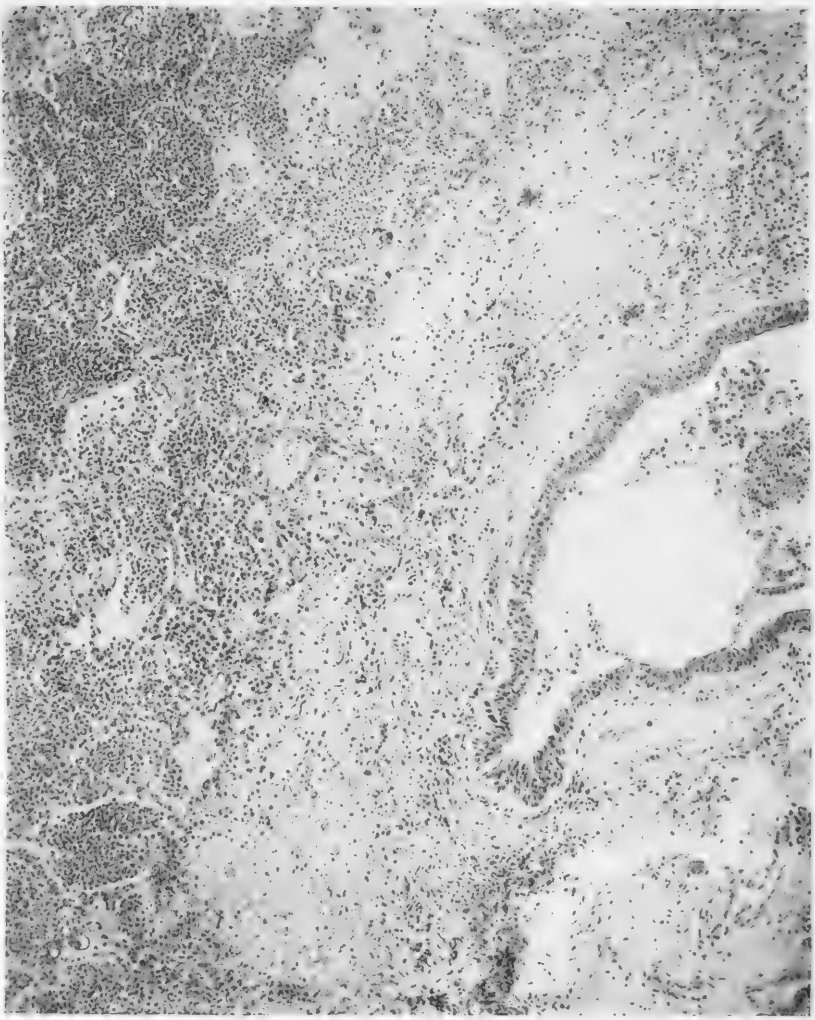


FIG. 11.

(Blake and Cecil: Experimental pneumonia. III.)

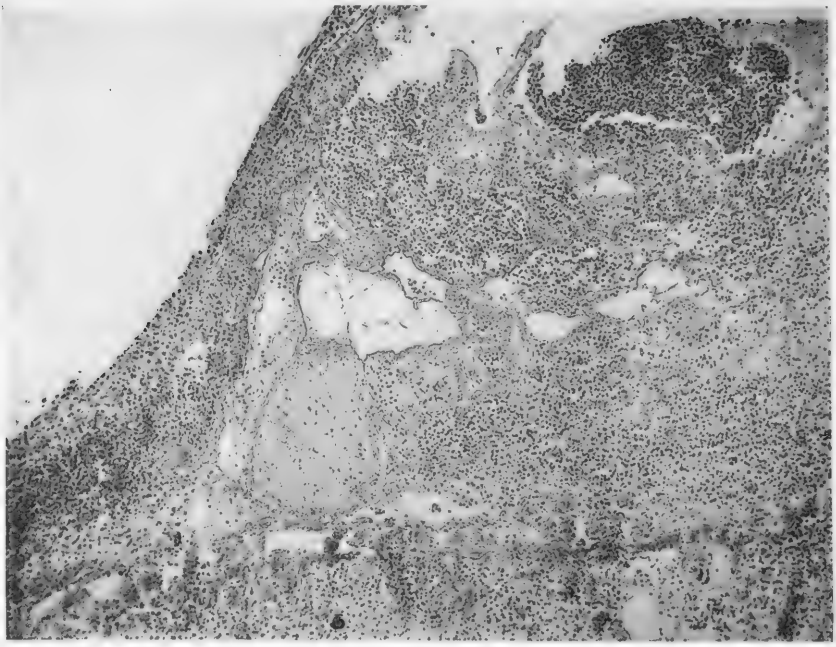


FIG. 12.

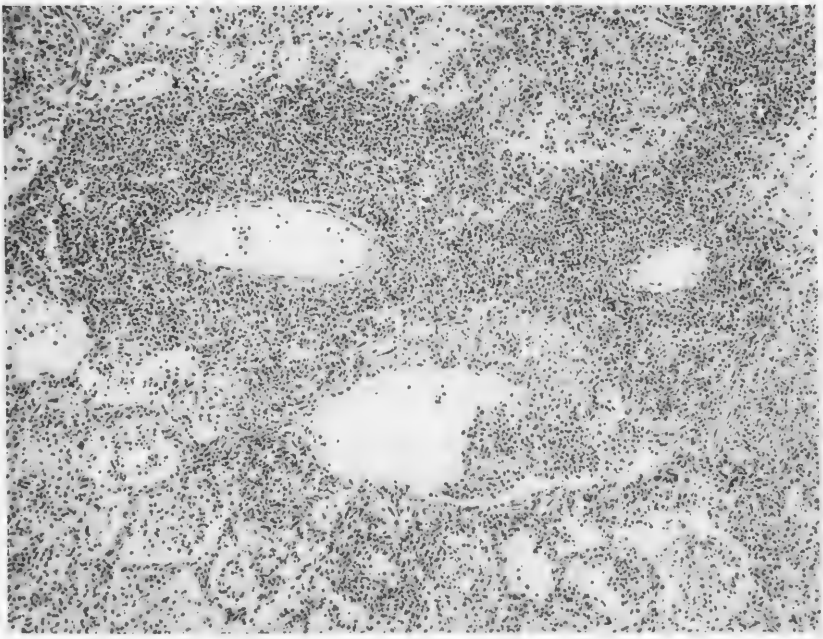


FIG. 13.

(Blake and Cecil: Experimental pneumonia. III.)

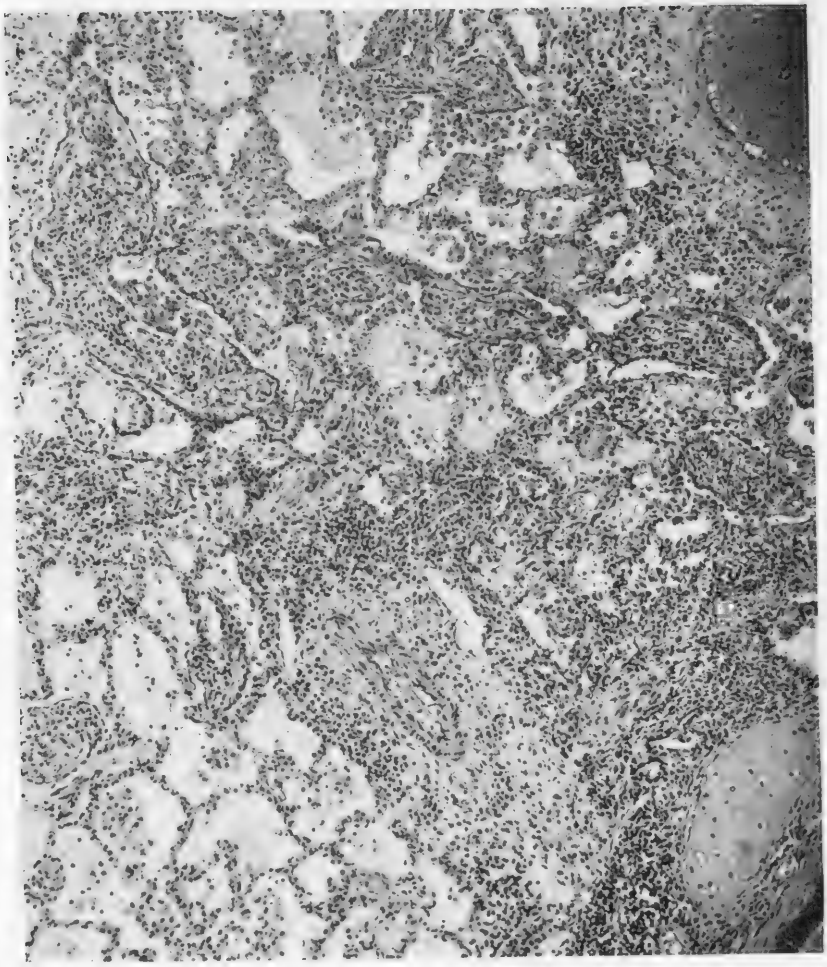


FIG. 14.

(Blake and Cecil: Experimental pneumonia. III.)

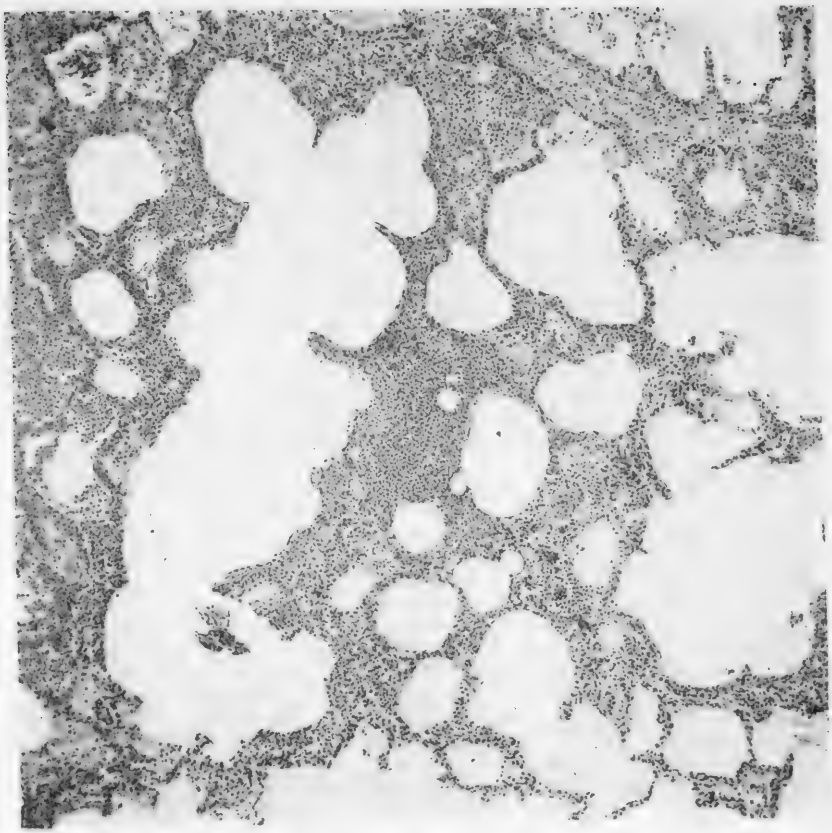


FIG. 15.

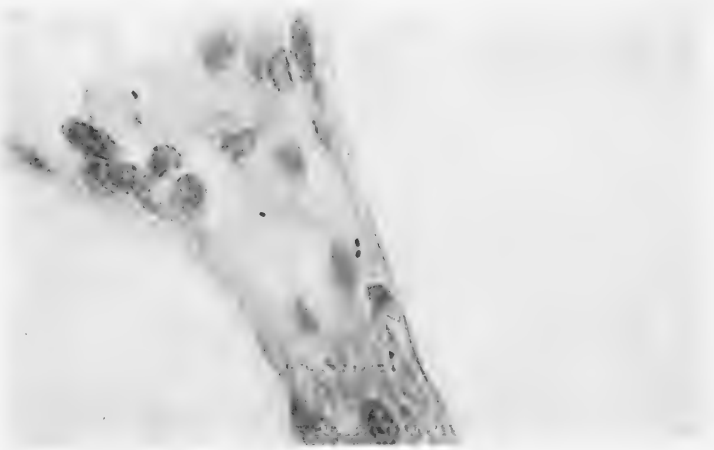


FIG. 16.

(Blake and Cecil: Experimental pneumonia. III.)

STUDIES ON EXPERIMENTAL PNEUMONIA.

IV. Results of Prophylactic Vaccination against Pneumococcus Pneumonia in Monkeys.

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(Received for publication, January 23, 1920.)

Prophylactic vaccination against pneumonia has been practised with apparent success on the miners in South Africa by Wright,[1] and more recently by Lister.[2] Within the past 2 years similar investigations have been undertaken in the United States Army Camps by Cecil and Austin[3] at Camp Upton, and by Cecil and Vaughan[4] at Camp Wheeler. The results obtained in these later experiments were so encouraging that the whole question of active immunity against pneumococcus seemed worthy of thorough study.

It has long been recognized that injection into animals of killed cultures of pneumococcus would protect them against lethal doses of living virulent pneumococci injected intravenously. Animals vaccinated in this way usually develop agglutinins and protective substances in their sera. Dochez[5] has shown that in man protective bodies are usually demonstrable in the serum of a patient immediately following an attack of pneumonia; and Cecil and Austin[3] found that the injection of killed pneumococci in man would, in some cases at least, stimulate the production of agglutinins and protective bodies.

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[1] Wright, A. E., *Lancet*, 1914, i, 87.

[2] Lister, F. S., An experimental study of prophylactic inoculation against pneumococcal infection in the rabbit and in man, Publications of the South African Institute for Medical Research, No. 8, Johannesburg, 1916; Prophylactic inoculation of man against pneumococcal infections, and more particularly against lobar pneumonia, Publications of the South African Institute for Medical Research, No. 10, Johannesburg, 1917.

[3] Cecil, R. L., and Austin, J. H., *J. Exp. Med.*, 1918, xxviii, 19.

[4] Cecil, R. L., and Vaughan, H. F., *J. Exp. Med.*, 1919, xxix, 457.

[5] Dochez, A. R., *J. Exp. Med.*, 1912, xvi, 665.

In spite of the fact that the injection of killed cultures of pneumococcus in rabbits, horses, and other animals will protect these animals against lethal doses of the living organism, this accomplishment is not equivalent to preventing the disease, pneumonia, itself. Indeed, very little is known concerning the whole subject of active immunity against pneumonia and comparatively little experimental work has been done along this line.

Wadsworth[6] immunized eleven rabbits against pneumococcus by injecting them with pneumococci which had been dissolved in rabbit bile. The eleven immunized rabbits and five controls were then injected intratracheally with 1 cc. of a virulent pneumococcus culture. Of the five control animals, three died in 48 hours without lung lesions; a fourth lived 4 days and a small patch of pneumonia was found at autopsy. The fifth was dying on the 5th day when it was killed. A small area of consolidation was found in this animal also. Of the eleven immunized animals, none died, but a few were seriously ill from 24 to 36 hours. All the immunized animals, when killed, showed more or less extensive pulmonary consolidation.

In the experiments referred to above, rather large doses of pneumococcus were used for infecting the animals, and it is probable that the controls were overwhelmed by the infection before there was an opportunity for pneumonia to develop. It is possible that if smaller doses had been employed for the intratracheal injections, Wadsworth would have produced pneumonia in the controls, and, on the other hand, the immunized animals would have escaped infection altogether.

The production experimentally of typical lobar pneumonia in monkeys affords an excellent method of testing the value of pneumococcus vaccine. It has been shown[7,8] that pneumococcus pneumonia in monkeys differs in no respect clinically or pathologically from pneumococcus pneumonia in man. An inflammation of the lungs can be produced in rabbits, dogs, and other laboratory animals by introducing virulent pneumococci or streptococci into the trachea, but animals injected in this manner do not run the typical course of lobar pneumonia as observed in man. The object of the present study has been to determine first the value of prophylactic pneumococcus vaccination in general, and secondly, the relative merits of the different types of pneumococcus vaccine that have been employed.

[6] Wadsworth, A., *Am. J. Med. Sc.*, 1904, cxxvii, 851.
[7] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.
[8] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 445.

Technique.

All the vaccine employed in this study was monovalent and was prepared from an old culture of Pneumococcus Type I which had been carried in the laboratory stock for several years. This organism appeared to have lost practically all its virulence. 1 cc. of a 24 hour broth culture had no effect whatever on a mouse when injected intraperitoneally. This was the same culture which was used at the Army Medical School in the preparation of pneumococcus vaccine for army camps.

The culture used for infecting the monkeys was a highly virulent Pneumococcus Type I, originally isolated from a case of lobar pneumonia. This organism killed a mouse in doses of 0.0000001 cc., and 0.00000001 cc. was usually lethal.

Method of Producing Experimental Pneumonia.—Experimental pneumonia was produced by introducing a small quantity of an 18 hour broth culture of pneumococcus (0.000001 to 1 cc.) with a Luer syringe directly into the trachea by the method previously described.[7] Symptoms of pneumonia developed 24 to 48 hours after injection. In testing for resistance to infection following pneumococcus vaccination, the animals were injected intratracheally, in most cases 2 to 4 weeks after vaccination.

Experiments with Pneumococcus Type I Lipovaccine.

The first vaccine to be experimented with was a Pneumococcus Type I lipovaccine which had been prepared at the Army Medical School according to the process described by Whitmore, Fennel, and Petersen.[9]

18 hour glucose broth cultures of Pneumococcus Type I were centrifuged in a Sharpless machine. The sediment was dried at 53°C. for 24 hours. This killed all the pneumococci. The dried sediment was then weighed and ground with steel balls for 24 hours.

[9] Whitmore, E. R., Fennel, E. A., and Petersen, W. F., *J. Am. Med. Assn.*, 1918, lxx, 427.

Finally, the dry powder was suspended in cottonseed oil containing 2 per cent lanolin and diluted to the desired strength.[10]

Dosage.—In order to make the results comparable with those in man only one injection of lipovaccine was administered to each monkey. As in man, the vaccine was injected subcutaneously, the abdominal wall being the site of inoculation.

Two series of monkeys were vaccinated; the first series received each the same dose that a man received of Pneumococcus Type I in the triple pneumococcus lipovaccine prepared by the Army Medical School; that is, 16 billion pneumococci, or 0.8 mg. of the dried bacteria. The second series received a dose proportional to their weight as compared with the weight of man. The average weight of a man is 70 kilos; the average weight of a Philippine monkey is 4 kilos. This series therefore received $\frac{1}{16}$ of man's dose, or 1 billion pneumococci (0.05 mg. of the dried bacteria). The lipovaccine was always diluted so that the monkey received 1 cc. of the oily suspension. In the first experiment a lipovaccine was used which was about 4 months old. In the remainder of the experiments, however, a freshly prepared vaccine was substituted.

Preliminary Test of Pneumococcus Lipovaccine.—The first attempt to test the efficacy of pneumococcus lipovaccine was carried out before the minimal infecting dose had been determined. As a result, the infecting dose which was employed in this experiment was 1 million times the size actually necessary to infect a normal monkey. The experiment is reported, however, as it illustrates certain differences between pneumonia in vaccinated and unvaccinated monkeys.

Experiment 1.—Three *Macacus syrichtus* monkeys were used in this experiment (Table I). Monkey 14 had received a large dose (16 billion) of Pneumococcus Type I lipovaccine, Monkey 17 a small dose (1 billion) of the same, while Monkey 27 served as a control. On Mar. 26, 1919, each of these three monkeys received 1 cc. of an 18 hour broth culture of Pneumococcus Type I intratracheally.

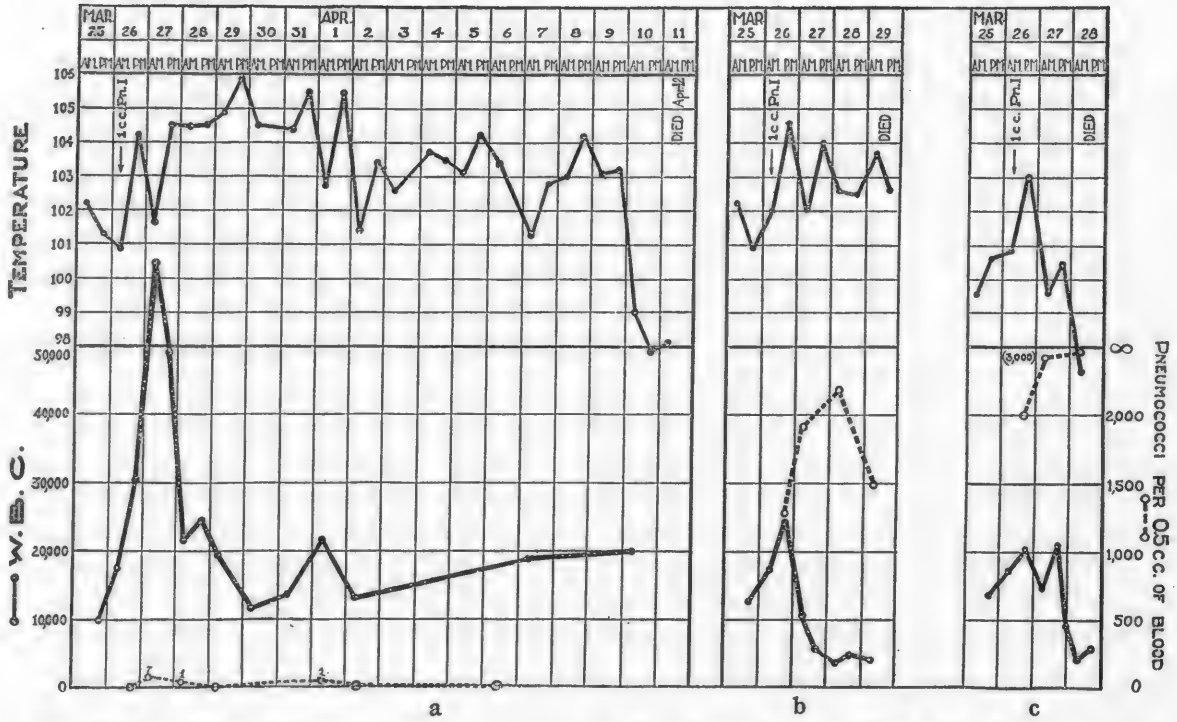
The results are shown in Table I and Text-fig. 1. All three monkeys died; the vaccinated monkeys, however, lived longer than the control. Monkey 14, which

[10] For full details for the method of preparing lipovaccine see articles by Whitmore, Fennel, and Petersen,[9] Whitmore and Fennel (Whitmore, E. R., and Fennel, E. A., *J. Am. Med. Assn.*, 1918, lxx, 1902), and Fennel (Fennel, E. A., *J. Am. Med. Assn.*, 1918, lxxi, 2115).

TABLE I.

Experimental Pneumonia Following Vaccination.

Monkey No.	Weight.	Mar. 11. Pn. I lipovaccine subcutaneously.	Mar. 25. Serum tests.		Mar. 26. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.				*cc.*				
14	2,850	0.8 mg. (16 billion).	0	0	1	Died in 17 days.	Resolving lobar pneumonia; hypertrophy and dilatation of heart.	Sterile.	Pn. I
17	2,800	0.05 mg. (1 billion).	0	0	1	" " 4 "	Lobar pneumonia; red stage.	Pn. I	" I
27	2,620	0	0	0	1	" " 3 "	Acute bronchitis; acute suppurative pericarditis (Pn. I).	Sterile.	" I (bronchus, Pn. I).



TEXT-FIG. 1, *a*, *b*, and *c*. Pneumococcus Type I pneumonia in vaccinated monkeys following the intratracheal injection of a large dose (1 cc.) of Pneumococcus Type I culture. (*a*) Monkey 14; vaccinated on Mar. 11 with 0.8 mg. (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*b*) Monkey 17; vaccinated on Mar. 11 with 0.05 mg. (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*c*) Monkey 27; control.

received a large dose of vaccine, had apparently recovered on Apr. 10, but died suddenly on Apr. 12 from a greatly dilated heart. The leucocyte reactions were more marked in the vaccinated monkeys, though there was not a great deal of difference between Monkeys 17 and 27. Finally, the blood culture in Monkey 14, which received a large dose of vaccine, remained practically sterile, while in the other two monkeys the blood contained large numbers of pneumococci. The control monkey was overwhelmed by the huge infecting dose and died before frank pneumonia developed. He showed, in addition to hemorrhagic bronchitis, acute suppurative pericarditis.

In this experiment vaccination failed to protect either monkey against pneumonia, but the results in the case of one vaccinated monkey suggested that vaccination had modified to some extent the virulence of the infection.

Effect of Small Doses of Pneumococcus Lipovaccine.—If protection against pneumococcus infection in monkeys could be obtained by vaccination, it was desirable to find the minimum efficient dose; in other words, a dose that would be comparable with that used in man. In the following experiment the vaccinated monkeys had each received a dose of lipovaccine proportional to their weight as compared with the weight of a man; that is, 0.05 mg. of the dried bacteria, or 1 billion pneumococci.

Experiment 2.—Six *Macacus syrichtus* monkeys were used in this experiment. Three (Monkeys 64, 65, and 67) had received 1 billion each of Pneumococcus Type I lipovaccine; the other three (Monkeys 85, 86, and 87) were controls (Table II). May 6, 1919. All six monkeys were injected intratracheally with an 18 hour broth culture of Pneumococcus Type I. Monkeys 65 and 87 received 0.1 cc.; Nos. 67 and 85 received 0.001 cc.; and Nos. 64 and 86 received 0.000001 cc.

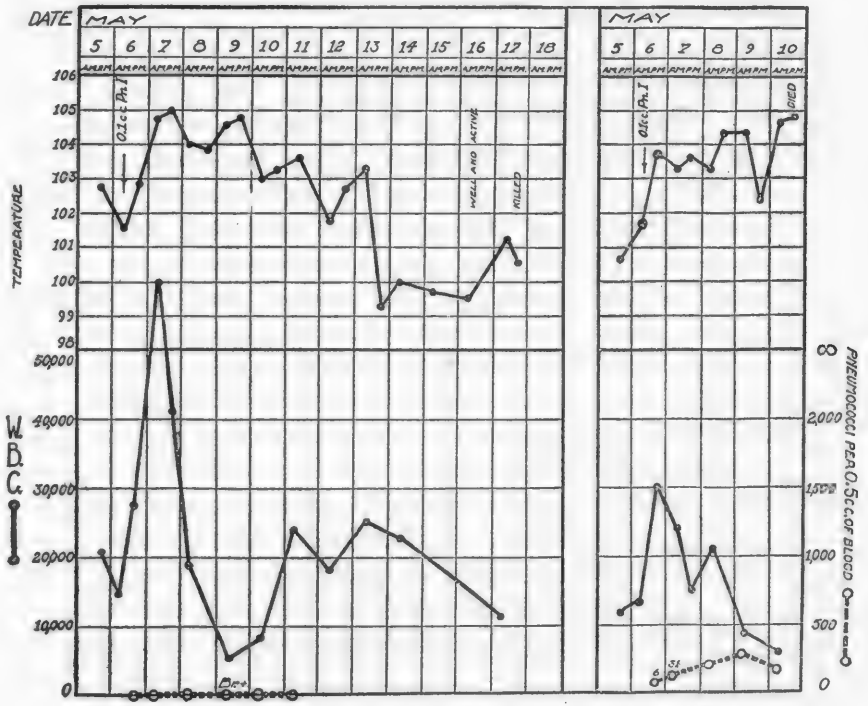
All six monkeys promptly developed symptoms of pneumonia. It will be observed, however, that while the three control monkeys died, two of the vaccinated monkeys recovered. The third vaccinated monkey (No. 67) had a crisis on the 9th day, but died suddenly on the 11th day of the disease. Autopsy revealed an old aortic endocarditis and insufficiency, with cardiac hypertrophy and dilatation. The size of the dose did not appear to exert a very pronounced influence on the course of the disease in either vaccinated or unvaccinated monkeys. Table II shows the protocols of these experiments, and Text-figs. 2, 3, and 4 exhibit the temperature, leucocyte, and blood culture curves.

TABLE II.

Results of Vaccination with a Small Dose of Pneumococcus Lipovaccine.

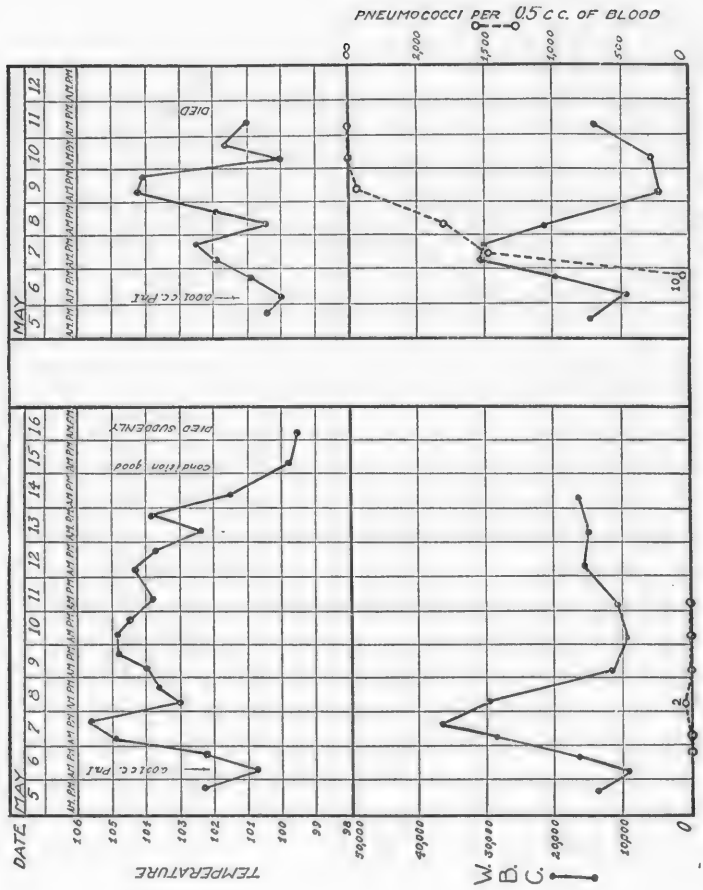
Monkey No.	Weight.	Apr. 21. Pn. I lipovaccine subcutaneously.	May 6. Serum tests.		May 6. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.				*cc.*				
65	3,075	0.05 mg. (1 billion).	0	0	0.1	Clinical pneumonia. Recovery by crisis on 8th day. Killed on 12th day.	Resolving lobar pneumonia, R. L.*	No growth.	No growth.
67	2,629	0.05 mg. (1 billion).	0	0	0.001	Clinical pneumonia. Crisis on 9th day. Died on 11th day.	Resolving lobar pneumonia; chronic aortic endocarditis and insufficiency; cardiac hypertrophy and dilatation.	Pn. I	Pn. I
64	2,599	0.05 mg. (1 billion).	0	0	0.000001	Clinical pneumonia. Recovery by lysis on 26th day. Killed on 30th day.	Organizing lobar pneumonia, L. L.	" I (4 colonies).	No growth.
87	2,835	0			0.1	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, L. U., L. M., L. L.; red stage.	Pn. I	Pn. I
85	2,675	0			0.001	Clinical pneumonia. Died on 6th day.	Lobar pneumonia, L. M., L. L.	" I	" I
86	2,424	0			0.000001	Clinical pneumonia. Died on 14th day.	Lobar pneumonia, R. L., L. L.; gray stage.	" I	No growth.

*L. U., L. M., L. L., etc., indicate lobes of the lung. The cardiac lobe is included as part of the right lower lobe.

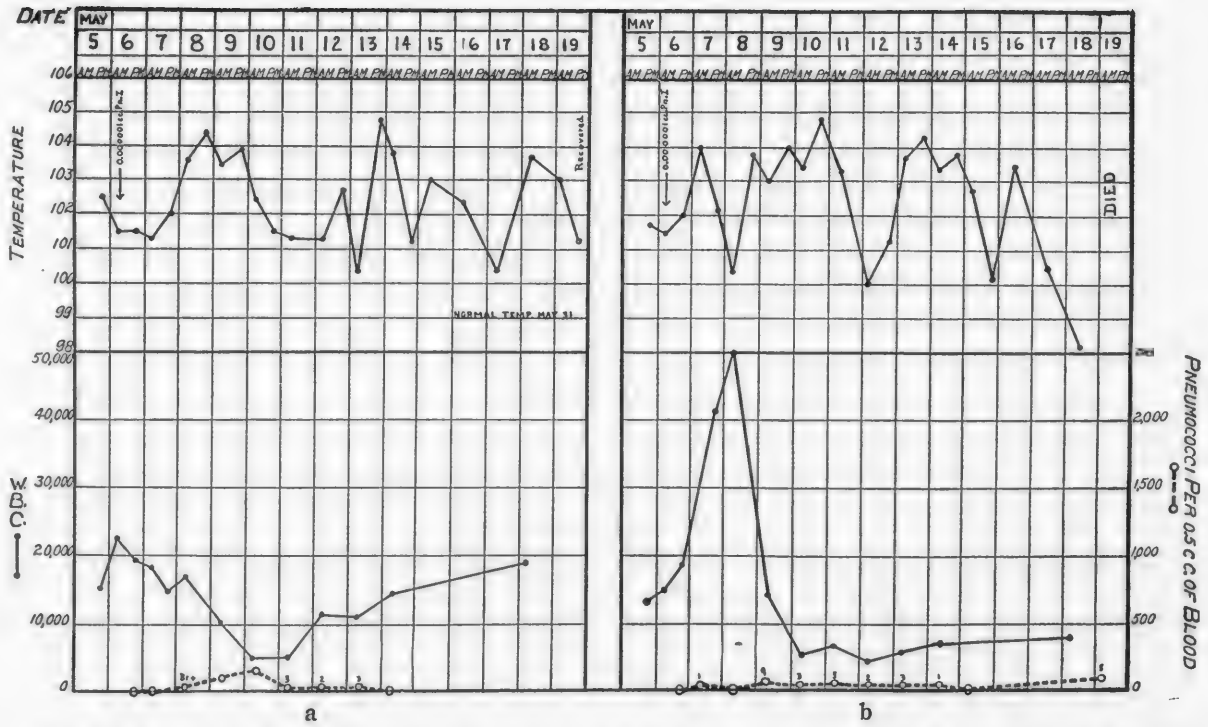


a b

TEXT-FIG. 2, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a small dose (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 65; vaccinated on Apr. 21. (*b*) Monkey 87; control.



TEXT-FIG. 3, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a small dose (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 67; vaccinated on Apr. 21. (*b*) Monkey 85; control.



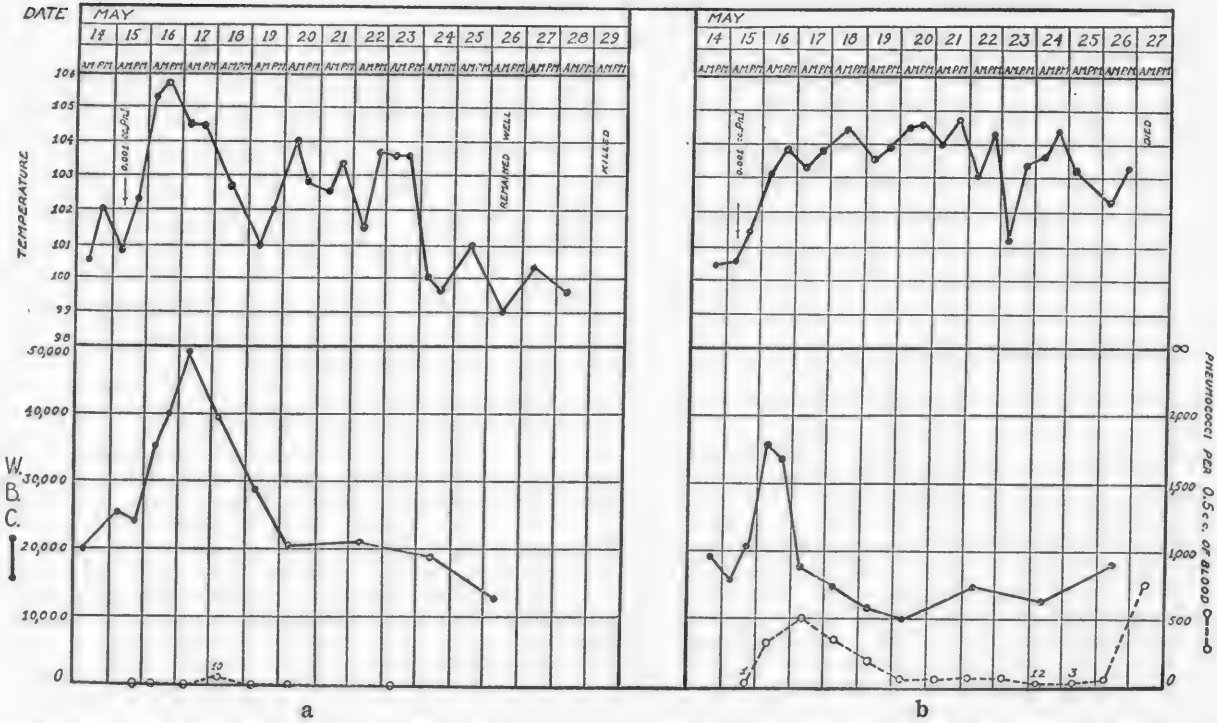
TEXT-FIG. 4, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a small dose (1 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 64; vaccinated on Apr. 21. (*b*) Monkey 86; control.

In these experiments, as in Experiment 1, it will be observed that the blood culture was sterile or weakly positive in vaccinated monkeys, while all the controls showed persistently positive blood cultures. These charts, however, demonstrate clearly that small doses of pneumococcus lipovaccine do not protect monkeys against pneumococcus pneumonia, even when the infecting dose is very small. It will also be observed that lipovaccine failed to stimulate agglutinins or protective substances in the monkeys' blood. Nevertheless, vaccination did appear to influence favorably the course of the disease.

Effect of Large Doses of Pneumococcus Lipovaccine.—In view of the failure of small doses of lipovaccine to protect against pneumonia, the next step was to determine the effect of large doses of pneumococcus lipovaccine. This experiment was carried out in the same manner as the one just described.

Experiment 3.—Six *Macacus syrichtus* monkeys were tested in this experiment (Table III, Text-figs. 5, 6, and 7). Monkeys 78, 80, and 81 had been vaccinated, each with 16 billion pneumococci. Monkeys 93, 95, and 96 were used for controls. All six monkeys were inoculated intratracheally with an 18 hour broth culture of Pneumococcus Type I. Monkeys 81 and 95 received 0.00001 cc., and Monkeys 80 and 96 received 0.000001 cc. on May 13, 1919. Monkeys 78 and 93 received 0.001 cc. on May 15. All six monkeys developed pneumonia. In this experiment two of the controls and two of the vaccinated monkeys recovered, while one in each series died. The death of the vaccinated monkey was unquestionably due to the complicating pericarditis which was discovered at autopsy.

As in Experiment 2, the vaccinated monkeys showed sterile or weakly positive blood cultures, with the exception of Monkey 80, in which the development of pericarditis probably contributed to the production of a fairly heavy blood infection. The character of the leucocyte reaction did not appear to be influenced by vaccination, nor was the disease appreciably shortened in the vaccinated group.



TEXT-FIG. 5, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 78; vaccinated on Apr. 29. (*b*) Monkey 93; control.

TABLE III.

Results of Vaccination with a Large Dose of Pneumococcus Lipovaccine.

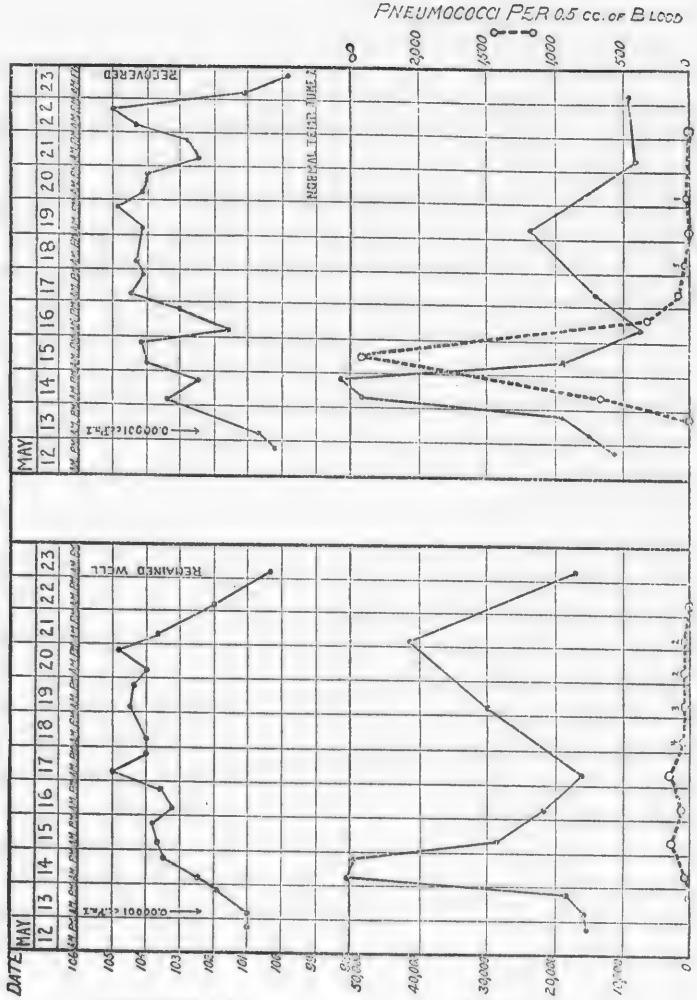
Monkey No.	Weight.	Apr. 29. Pn. I lipovaccine subcutaneously.	May 13. Serum tests.		May 13. Broth culture of Pn. I intratracheally.	May 15. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.		
			Agglutinins.	Protection.					Lung.	Heart's blood.	Pericardium.
	gm.				*cc.*	*cc.*					
78	5,322	0.8 mg. (16 billion).	0	0		0.001	Clinical pneumonia. Recovery by crisis on 10th day. Killed on 15th day.	Resolving lobar pneumonia, R. L., L. M.	No growth.	No growth.	
81	2,750	0.8 mg. (16 billion).	0	0	0.00001		Clinical pneumonia. Recovery by lysis on 10th day. Killed on 17th day.	Resolving lobar pneumonia, R. M., R. L.	" "	" "	
80	2,850	0.8 mg. (16 billion).	0	0	0.000001		Clinical pneumonia. Died on 12th day.	Lobar pneumonia, L. L., L. M.; acute pericarditis.	" I	Pn. I	Pn. I
93	5,110	0				0.001	Clinical pneumonia. Died on 13th day.	Lobar pneumonia; gray stage; entire right lung.	" I	" I	

95	4,110	0			0.00001		Clinical pneumonia. Crisis on 11th day. Relapse. Recovery on 21st day. Killed on 21st day.	Lobar pneumonia, L. L.; resolution and organization.	No growth.	No growth.
96	2,655	0			0.000001		Clinical pneumonia. Recovery by crisis on 8th day. Blood culture, Pn. I.			

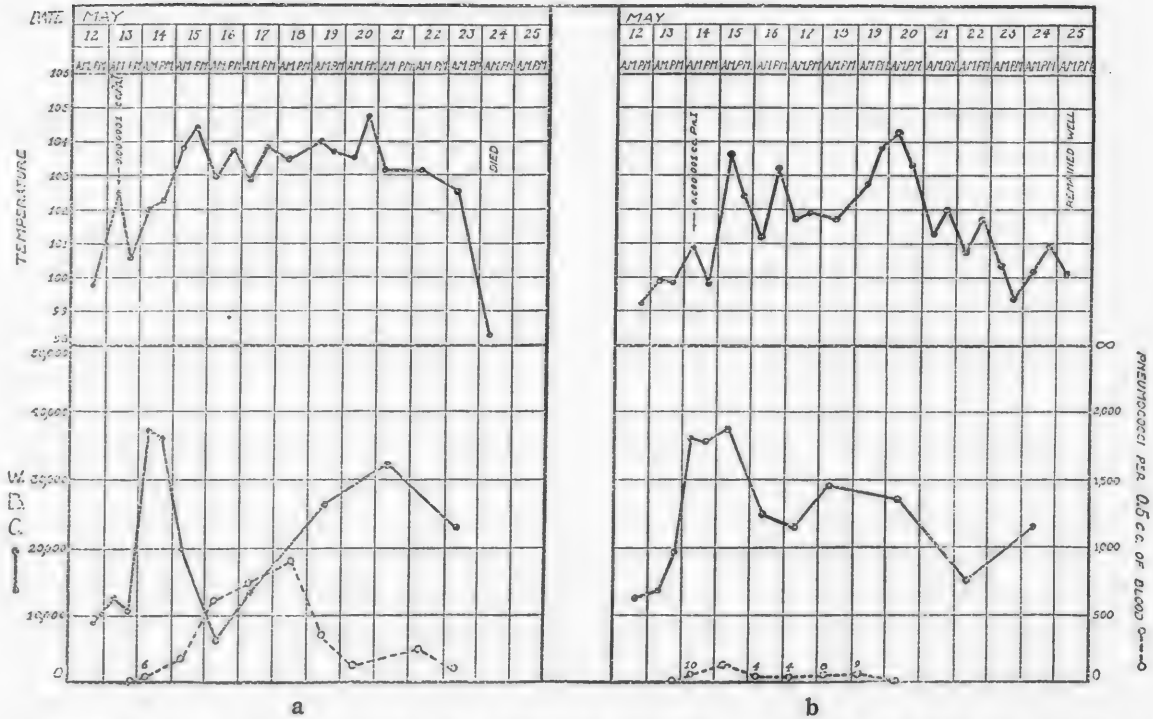
TABLE IV.

Spontaneous Pneumonia Following Vaccination.

Monkey No.	Weight.	Mar. 11. Pn. I lipovaccine subcutaneously.	Mar. 25. Serum tests with Pn. I.		Date of death.	Autopsy.	Autopsy cultures.		
			Agglutinins.	Protection.			Lung.	Heart's blood.	Pericardium.
	gm.				*1919*				
15	4,000	0.05 mg. (1 billion).	0	0	Apr. 9	Lobar pneumonia; stage of resolution.	Pn. IV	Sterile.	
13	5,055	0.05 mg. (1 billion).	0	0	" 25	Lobar pneumonia; gray stage; acute pericarditis.	Sterile.	Pn. IV	Pn. IV
21	2,815	0.05 mg. (1 billion).	0	0	" 29	Resolving lobar pneumonia; acute pericarditis.	"		Pn. (type undetermined).



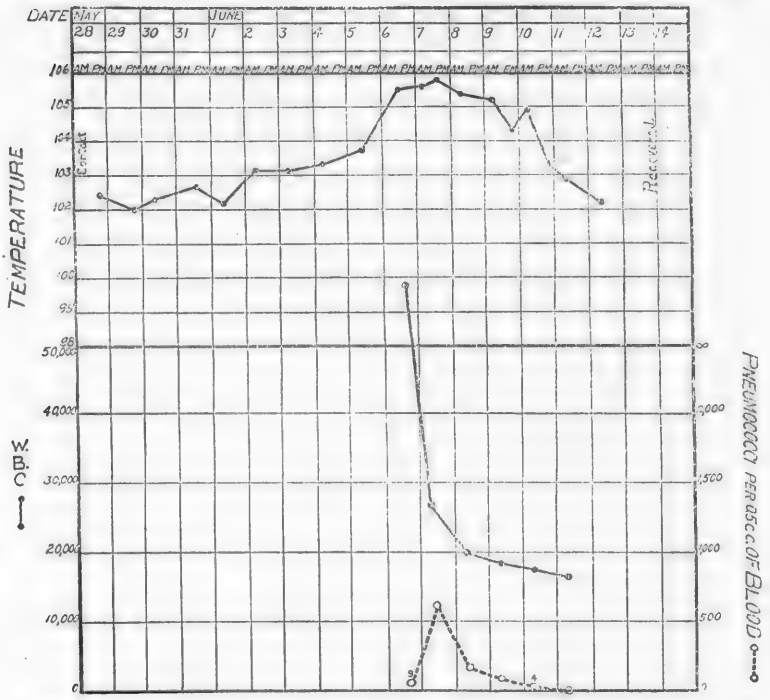
TEXT-FIG. 6, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 81; vaccinated on Apr. 29. (*b*) Monkey 95; control.



TEXT-FIG. 7, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. (*a*) Monkey 80; vaccinated on Apr. 29. (*b*) Monkey 96; control.

Contact Experiments.

From the preceding experiments it is evident that pneumococcus lipovaccine in the dosage employed failed to protect monkeys against experimental pneumonia. In order, however, to forestall the criticism which might be made that pneumonia had been produced by artificial means, it was decided to test the immunity of the vaccinated monkeys against spontaneous pneumonia by means of a contact test.



TEXT-FIG. 8. Spontaneous Pneumococcus Type I pneumonia developing in Monkey 16, vaccinated on Mar. 11 with a large dose (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. Infection followed contact in the same cage with another case of Pneumococcus Type I pneumonia.

Experiment 4.—Three *Macacus syrichtus* monkeys which had previously been vaccinated with pneumococcus lipovaccine were placed in a cage with three normal healthy monkeys. Two monkeys in the active stage of Pneumococcus

Type I pneumonia were then put in the cage with the other six monkeys and the eight animals kept in intimate contact for 2 weeks. A few days after the experiment was started, Monkey 16, one of the vaccinated animals, became ill with pneumonia. The protocol follows:

Mar. 11, 1919. Monkey 16, *Macacus syrichtus*; weight 3,630 gm. Received 0.8 mg. (16 billion) of Pneumococcus Type I lipovaccine subcutaneously. May 26. Monkey placed in the same cage with two other vaccinated monkeys, three normal monkeys and two monkeys suffering with Pneumococcus Type I pneumonia. June 6. Monkey appears sick; marked leucocytosis. Blood culture shows Pneumococcus Type I. June 7. Typical lobar pneumonia; blood culture shows 650 colonies of Pneumococcus Type I per 0.5 cc. of blood. June 11. Marked improvement. Monkey has run a typical course of lobar pneumonia.

This experiment shows that Monkey 16, in spite of having been vaccinated with a large dose of pneumococcus lipovaccine, was unable to resist infection with Pneumococcus Type I when exposed to pneumonia due to this type. It will be observed, however, in Text-fig. 8, that the disease ran a mild and fairly short course, which supports the observation previously made that vaccinated monkeys tolerate pneumonia more readily than unvaccinated animals. Strangely enough, another one of the vaccinated animals in the contact test developed pneumonia, but in this instance the infection proved to be with Pneumococcus Type IV.[11] None of the control monkeys became infected.

Spontaneous Pneumococcus Type IV Pneumonia in Monkeys Vaccinated against Pneumococcus Type I.—Some of the monkeys that had been inoculated with Pneumococcus Type I lipovaccine were put back into a large cage with a number of stock monkeys. An epidemic of Pneumococcus Type IV pneumonia broke out in this cage and a number of the vaccinated monkeys contracted the disease.

Experiment 5.—Three *Macacus syrichtus* monkeys (Nos. 13, 15, and 21) had received a small dose of Pneumococcus Type I lipovaccine (Table IV). Pneumonia developed spontaneously in all of them 4 to 7 weeks after vaccination, just at the time when presumably their immunity should have been at a high point. In Monkeys 13 and 15 Pneumococcus Type IV was recovered from the autopsy cultures, and in Monkey 21 a pneumococcus was seen in the pericardial fluid, but failed to grow in the culture.

[11] See Paper I.[7]

The protocols demonstrate the fact that monkeys vaccinated with Pneumococcus Type I lipovaccine possess no demonstrable cross-immunity against spontaneous Pneumococcus Type IV pneumonia.

Summary of Lipovaccine Experiments.—A review of the experiments so far reported brings out these facts:

1. Pneumococcus Type I lipovaccine, whether used in large or small doses has failed to protect monkeys against experimental and spontaneous Pneumococcus Type I pneumonia.

2. Vaccination, however, does appear to modify the course of a subsequent Type I pneumonia. The blood is not so heavily infected as in unvaccinated animals, in some cases remaining practically sterile throughout the entire course of the disease. Furthermore, the mortality rate is lower in the vaccinated monkeys and the disease seems to run a milder course.

3. No agglutinins or protective bodies were demonstrated in any of the monkeys inoculated with lipovaccine.

4. There is no evidence that Pneumococcus Type I lipovaccine confers any cross-immunity against other types of pneumococcus pneumonia.

Experiments with Pneumococcus Type I Saline Vaccine.

The failure of pneumococcus lipovaccine to protect monkeys against pneumonia prompted us to test the value of pneumococcus saline vaccine. Such experiments seemed all the more justified in view of the fact that the results of prophylactic vaccination against pneumonia at Camp Upton, where a saline vaccine had been used, were distinctly better than the results at Camp Wheeler where the lipovaccine had been employed.

The pneumococcus saline vaccine was prepared from the same avirulent strain of Pneumococcus Type I which had been used in the preparation of the Pneumococcus Type I lipovaccine. The saline vaccine was made as follows:

Pneumococci were cultivated for 18 hours in glucose broth and submitted to centrifugation. The bacterial sediment was then heated at 55°C. for 1 hour to kill the pneumococci. The vaccine was diluted with normal salt solution containing 0.25 per cent tricresol

and standardized by Wright's method. The saline vaccine used in the following experiments was prepared on May 1, and the experiments were started on May 6, 1919.

For the most part, the dosage and method of administration in the experiments with pneumococcus saline vaccine were the same as in the lipovaccine tests. In the following experiments each monkey received only one subcutaneous injection.

Results of Vaccination with Pneumococcus Type I Saline Vaccine.—In testing the saline vaccine the effect of the large and small dosage was determined in one experiment.

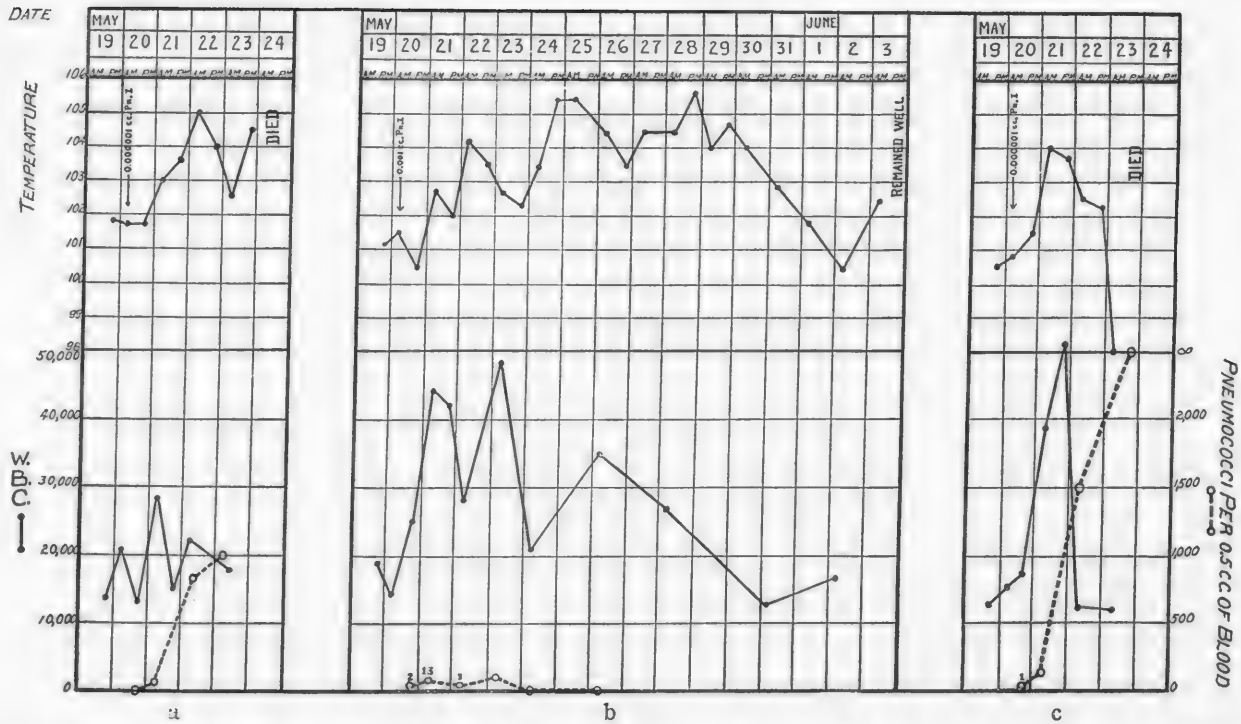
Experiment 6.—Four *Macacus syrichtus* monkeys were used in this experiment (Table V, Text-figs. 9 and 10). Monkeys 88 and 89 had each been vaccinated with 1 billion, Monkey 90 with 16 billion Pneumococcus Type I saline vaccine. Monkey 98 was the control. 2 weeks after these monkeys were vaccinated, their blood was tested for agglutinins and protective bodies. No agglutinins could be demonstrated, but all three monkeys showed the presence of protective bodies. In Monkeys 89 and 90 the protection was marked, in Monkey 88 slight. 2 weeks after vaccination the monkeys were injected intratracheally with an 18 hour broth culture of Pneumococcus Type I. Monkeys 88, 90, and 98 received each 0.000001 cc. of culture. Monkey 89 received 0.001 cc. of culture. The results are shown in Table V. While the four monkeys all developed pneumonia, the control monkey ran a rapid course and died on the 4th day. The vaccinated animals lived longer and two of them recovered (Monkeys 89 and 90). Monkey 88 died on the 5th day. The two cases that terminated fatally showed extensive lobar pneumonia at autopsy and Pneumococcus Type I was recovered from the lungs and heart's blood. One of the vaccinated monkeys that recovered was killed and at autopsy showed a resolving pneumonia, cultures from which were sterile. The temperature, leucocyte, and blood culture curves are shown in Text-figs. 9 and 10.

With pneumococcus saline vaccine as with lipovaccine, prophylactic inoculation failed to protect monkeys against pneumonia, but, as in the case of lipovaccine, inoculation seemed to modify favorably the course of the disease. The vaccinated monkey that died (Monkey 88) was the one which showed the smallest amount of protective substances in its blood. The two vaccinated monkeys which recovered showed only a moderate degree of bacteremia, whereas the two monkeys that died had heavy blood infections. The amount of pneumococcus culture used for infecting the monkeys appears to have

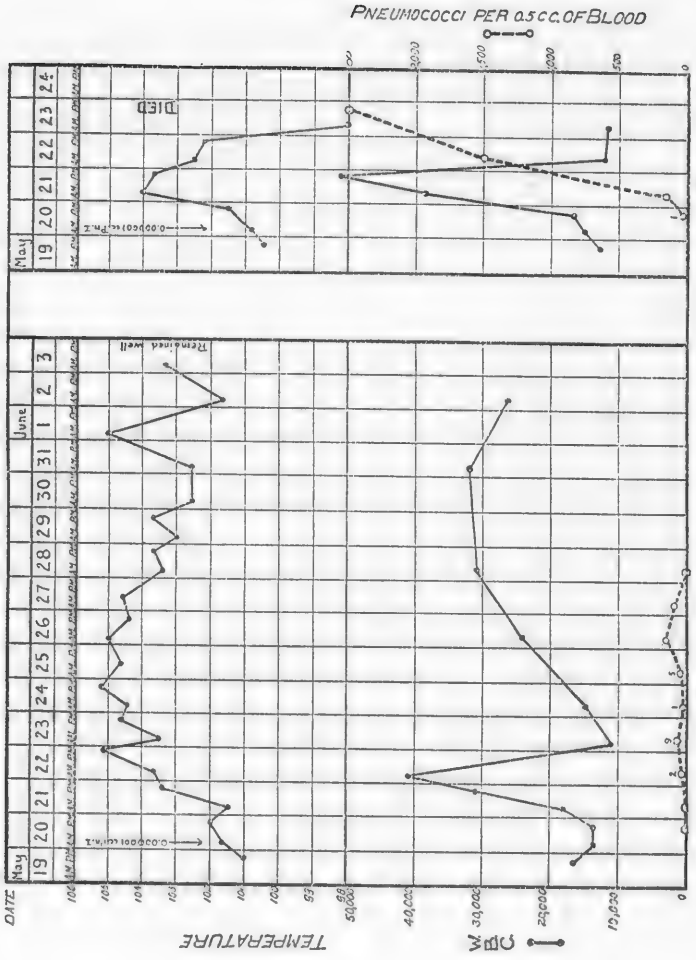
TABLE V.

Results of Vaccination with Pneumococcus Saline Vaccine.

Monkey No.	Weight.	May 6. Pn. I saline vaccine subcutaneously.	May 19. Serum tests.			May 20. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
			Agglutinins.	Protection.	Control.				Lung.	Heart's blood.
	gm.					*cc.*				
88	2,072	1 billion.	0	0.00001 cc. Died in 60 hrs. 0.000001 cc. Died in 60 hrs. 0.0000001 cc. Survived.	0.00001 cc. Died in 24 hrs. 0.000001 cc. Died in 48 hrs. 0.0000001 cc. Died in 48 hrs.	0.000001	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, entire left lung, R. L.; red stage.	Pn. I	Pn. I
89	2,462	1 "	0	0.00001 cc. Survived. 0.000001 cc. Survived. 0.0000001 cc. Survived.		0.001	Clinical pneumonia. Recovery by lysis on 12th day.	Resolving lobar pneumonia, R. L.	No growth.	No growth.
90	3,922	16 "	0	0.00001 cc. Survived. 0.000001 cc. Survived. 0.0000001 cc. Survived.		0.000001	Clinical pneumonia. Recovery by lysis on 11th day.			
98	4,100	0				0.000001	Clinical pneumonia. Died on 4th day.	Lobar pneumonia, R. U., R. L., L. U., L. L.; stage of engorgement.	Pn. I	Pn. I



TEXT-FIG. 9, *a*, *b*, and *c*. Pneumococcus Type I pneumonia in monkeys vaccinated with a small dose (1 billion) of Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 88; vaccinated on May 6. (*b*) Monkey 89; vaccinated on May 6. (*c*) Monkey 98; control.



TEXT-FIG. 10, *a* and *b*. Pneumococcus Type I pneumonia in a monkey vaccinated with a large dose (16 billion) of Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 90; vaccinated on May 6. (*b*) Monkey 98; control.

little influence, up to a certain point, upon the course of the disease. Monkey 88 received only 0.000001 cc. and died on the 5th day. Monkey 89 received 0.001 cc. (a thousand times as large a dose) and recovered from the infection after running a comparatively mild course.

Results of Vaccination with Three Injections of Pneumococcus Type I Saline Vaccine.—Although one injection of pneumococcus saline vaccine failed to protect monkeys against pneumococcus infection, the results were rather more encouraging than those obtained with the lipovaccine. In two of the vaccinated monkeys tested, a high degree of protection was demonstrable in the blood following the inoculation, and although these two monkeys both contracted pneumonia, the disease ran a mild course and both monkeys recovered. Therefore it seemed desirable to determine whether three injections of pneumococcus saline vaccine given at intervals of 1 week would not afford the necessary amount of protection.

Experiment 7.—This series of *Macacus syrichtus* monkeys was started on saline vaccine May 22, 1919, each monkey receiving weekly subcutaneous injections of 1 billion pneumococci until three inoculations had been given. 2 weeks after the third injection the blood from these monkeys was tested for agglutinins and protective bodies. No agglutinins or protective bodies could be demonstrated in any of the five monkeys tested.

June 20. Two of the vaccinated monkeys and a control monkey were injected intratracheally with 0.000001 cc. of an 18 hour broth culture of Pneumococcus Type I. Table VI and Text-fig. 11 show the results obtained. All three monkeys developed pneumonia, and in all three the disease was fatal. In one vaccinated monkey (Monkey 100) the disease presented features which are usually associated with a mild attack; namely, a moderate infection of the blood and a good secondary rise in the leucocytes, while in the other (Monkey 101) a septicemia equally as heavy as that of the control developed. At autopsy all three monkeys showed lobar pneumonia, and Pneumococcus Type I was recovered from the organs.

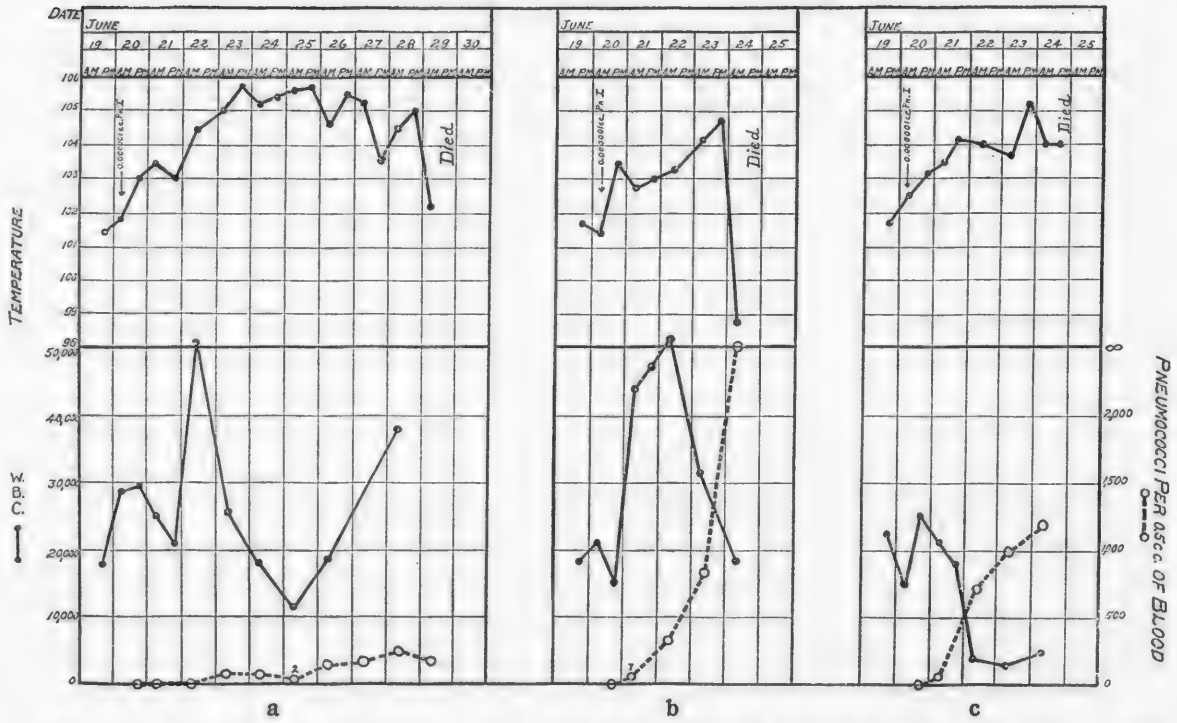
In this experiment three small doses of saline vaccine failed to give as much protection as had been obtained by a large single injection in the previous experiments.

Summary of Saline Vaccine Experiments.—The experiments which have been reported indicate that saline vaccine like lipovaccine, when injected subcutaneously in moderate doses, has failed to protect

TABLE VI.

Results of Vaccination with Three Injections of Pneumococcus Saline Vaccine.

Monkey No.	Weight.	Pn. I saline vaccine subcutaneously.			June 20. Serum tests.		June 20. Broth culture of Pn. I intratracheally.	Result.	Autopsy.	Autopsy cultures.	
		May 22.	May 29.	June 5.	Agglutinins.	Protection.				Lung.	Heart's blood.
	gm.						*cc.*				
100	2,392	1 billion.	1 billion.	1 billion.	0	0	0.000001	Clinical pneumonia. Died on 10th day.	Lobar pneumonia, L. U., L. M., L. L., R. L.; gray stage.	No growth.	Pn. I
101	2,532	1 "	1 "	1 "	0	0	0.000001	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, L. U., L. M., L. L., R. L.; stage of engorgement.	Pn. I	" I
114 (control).	3,000	0	0	0			0.000001	Clinical pneumonia. Died on 5th day.	Lobar pneumonia, L. L., R. L.; stage of engorgement.	" I; *S. hæmolyticus.*	" I; *S. hæmolyticus.*



TEXT-FIG. 11, *a*, *b*, and *c*. Pneumococcus Type I pneumonia in monkeys vaccinated with three injections (1 billion each) of Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 100. (*b*) Monkey 101. Both these animals received vaccine on May 22, 29, and June 5. (*c*) Monkey 114; control.

monkeys against pneumococcus pneumonia. As for any relative superiority of one vaccine over the other, there appears to be little choice between the two. Saline vaccine, however, is more likely to stimulate the formation of protective bodies in the blood, and for this reason probably gives a somewhat better immunity. The individual variation in the natural resistance of monkeys to pneumococcus infection is a factor of primary importance and one which must always be considered. No definite decision can be reached as to the relative merits of pneumococcus lipovaccine and saline vaccine, except by testing a large series of monkeys with each type.

Effect of Intravenous Injection of Living Pneumococcus Type I Cultures in Monkeys Vaccinated with Pneumococcus Type I Vaccine.

In the preceding experiments it has been shown that pneumococcus vaccine does not protect monkeys against intratracheal infection with pneumococcus. It seemed desirable, therefore, for the sake of comparison, to determine whether these vaccinated monkeys would be protected against intravenous infection.

Experiment 8.—July 8, 1919. Two *Macacus syrichtus* monkeys that had been vaccinated 4 weeks previously with three injections (1 billion each) of Pneumococcus Type I saline vaccine and one control monkey were injected intravenously with 0.001 cc. of a broth culture of living virulent Pneumococcus Type I. This dose is often fatal for a normal monkey. The results are shown in Table VII and Text-fig. 12. The vaccinated monkeys showed few or no clinical symptoms following the injection. Monkey 105 remained perfectly well, with sterile blood cultures. Monkey 104, the other vaccinated monkey, had a mild febrile reaction and a temporary infection of the blood of 48 hours duration. The control (Monkey 116) was ill for 6 days with high fever and heavy septicemia.

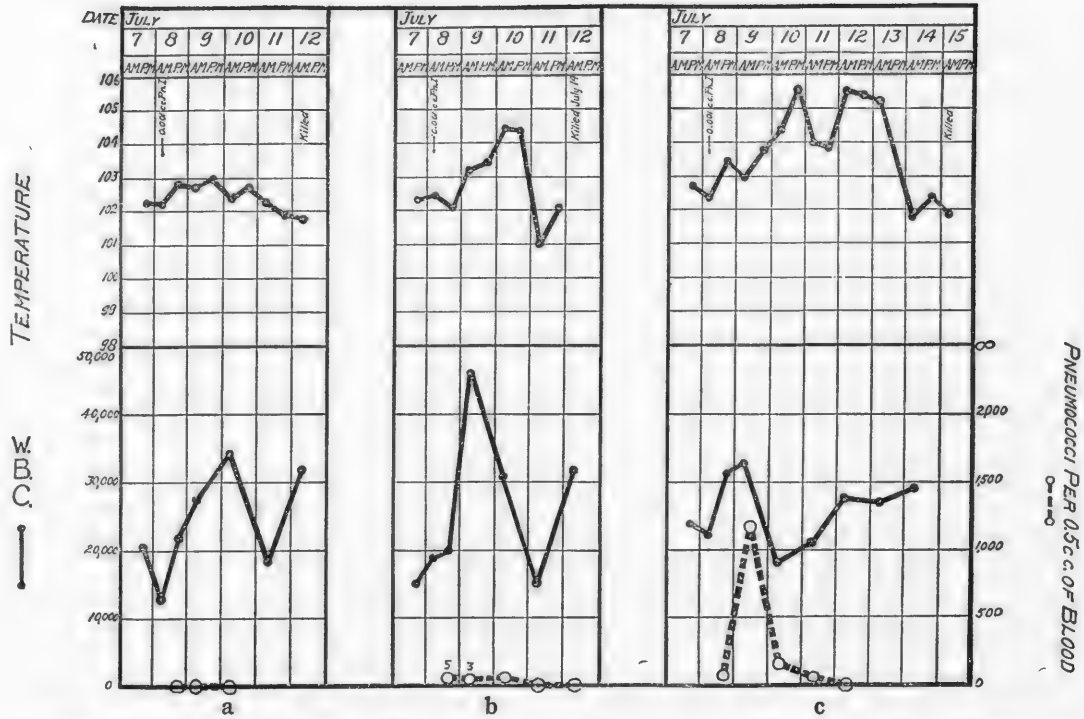
All three monkeys were killed just after their temperature had returned to normal, and all showed perfectly normal lungs.

This experiment has considerable significance in suggesting that distinction must be made between a humoral immunity against pneumococcus and a local immunity, possibly cellular, in the lungs. The same dose of culture which Monkeys 104 and 105 received intravenously with impunity would have produced a severe pneumonia if administered intratracheally. Although this phenomenon may at

TABLE VII.

Effect of Intravenous Injection of Virulent Pneumococcus Type I in Monkeys Previously Vaccinated with Pneumococcus Type I Saline Vaccine.

Monkey No.	Weight.	Pn. I saline vaccine subcutaneously.			June 21. Serum tests.		July 8. Broth culture of Pn. I intravenously.	Result.	Autopsy.	Autopsy cultures. Heart's blood.
		May 22.	May 29.	June 5.	Agglutinins.	Protection.				
	gm.						*cc.*			
105	1,375	1 billion.	1 billion.	1 billion.	0	0	0.001	No clinical symptoms; no septicemia. Killed on 5th day.	Normal lungs.	No growth.
104	1,735	1 "	1 "	1 "	0	0	0.001	Fever for 2 days; mild septicemia for 2 days; no symptoms of pneumonia. Killed on 7th day.	" "	" pneumococci.
116 (control).	1,935	0	0	0			0.001	Fever for 6 days; heavy septicemia; no symptoms of pneumonia. Killed on 8th day.	" "	No growth.



TEXT-FIG. 12, *a*, *b*, and *c*. Reactions to intravenous injection of living virulent Pneumococcus Type I in monkeys previously vaccinated with Pneumococcus Type I saline vaccine subcutaneously. (*a*) Monkey 105. (*b*) Monkey 104. Both these animals received vaccine on May 22, 29, and June 5. (*c*) Monkey 116; control.

first seem paradoxical, it is in reality not out of harmony with the results obtained in the preceding experiments in which it has been clearly shown that prophylactic vaccination prevented to a large extent the development of septicemia during the course of lobar pneumonia. It furthermore demonstrates that tests of the prophylactic value of vaccination in animals may lead to false conclusions if these depend upon the demonstration of immunity to intravenous infection rather than to the actual disease against which the vaccination is directed.

DISCUSSION.

In this study all efforts to protect monkeys against pneumonia by subcutaneous vaccination with killed cultures of pneumococcus have failed. With our present ignorance concerning the nature of bacterial antigen, any investigation of this nature must necessarily be for the most part empirical. In the experiments reported, two kinds of vaccine have been tried—the oily and the saline. But obviously, many other factors determine the character of a vaccine, each of which should be considered in determining the value of the vaccine for prophylactic inoculation. Among these factors are the following:

Virulence of the Organism.—It has generally been assumed that a virulent organism produces a more efficient vaccine than an avirulent strain. The virulent strain, however, causes a more severe reaction, and for this reason most vaccines in general use are prepared from avirulent cultures. The question of whether a vaccine prepared from a highly virulent pneumococcus will afford better protection than one prepared from an avirulent strain has not been attacked in the present study. It may, however, be a more important factor than is generally assumed and it is hoped that this question can be investigated at a later time.

Method of Cultivation.—When vaccines are prepared from cultures grown on solid media, all the products of bacterial metabolism are presumably included in the vaccine. When, however, the bacteria are grown in liquid media, the supernatant fluid is discarded, and with it the bacterial metabolites. Just how much antigenic value these products have is problematical. It is obvious, moreover, that in vaccines prepared from broth media, the longer the period of incu-

bation, the greater the autolysis and the greater the amount of bacterial products in solution in the broth. The influence of sugars, animal sera, etc., when added to the culture medium may be an important factor in the quality of a vaccine.

Method of Killing the Bacteria.—Many methods have been used for killing the bacteria in vaccines, but heat and germicides remain the two most frequently employed. In the case of the lipovaccine and saline vaccine used in the present study the pneumococci were killed by heat; but in the lipovaccine the bacteria were heated for 24 hours at 53°C., whereas in the preparation of the saline vaccine they were heated for only 1 hour at 55°C. Whether this difference in the duration of heating had any effect on the antigenic value of the vaccine, it is impossible to say.

Vehicle in Which the Bacteria Are Suspended.—Until quite recently all vaccines were prepared in either water or normal salt solution. The employment of vegetable oils as a vehicle for suspending bacteria introduces a new factor into the question of antigenic value. It is possible that in oily vaccines a capsule forms around the body of the microorganism and interferes not only with its absorption but also with the production of specific antibodies. In these experiments monkeys injected with pneumococcus lipovaccine failed to develop demonstrable agglutinins and protective bodies.

Age of the Vaccine.—The age of the vaccine is undoubtedly an important factor in its antigenic value and one that has not been thoroughly investigated. The lipovaccine used in the first of these experiments was 4 months old at the time the experiment was started. In the later experiments a fresh lipovaccine was used, prepared in the same way as the older one, and no difference in effect was observed in the two vaccines. With the saline vaccine, the first experiment, which was carried out immediately after the vaccine was prepared, showed protective bodies in the three monkeys tested. A month later the same vaccine was used for testing the effect of three repeated injections of saline vaccine and no protective bodies could be demonstrated in any of the five monkeys vaccinated. Whether this was a matter of individual variation in the monkeys or whether there occurred certain changes in the vaccine due to standing 1 month in the ice box, it is hard to say. The former hypothesis would appear more reasonable.

The results obtained in this study of prophylactic vaccination against pneumonia in monkeys have been disappointing; but it should be borne in mind that the test applied has been a particularly crucial one. Comparatively small doses of vaccine have been used in order to make the results comparable with vaccination in man. No doubt a satisfactory immunity could have been obtained if repeated injections of large doses of vaccine had been administered, and still better results might have been reached if, in the case of saline vaccine, the injections had been given intravenously. Such an accomplishment, however, was not the aim of the investigation.

Furthermore, the Type I pneumococcus which was employed in the intratracheal injections was an organism of extraordinary virulence. It practically never failed to produce the disease even in doses of 0.000001 cc. of broth culture, and in unvaccinated monkeys the result was usually fatal. One hundred millionth of a cc. was in most cases lethal for a mouse.

Finally, it must be emphasized that the monkey is highly susceptible to the pneumococcus. The prevalence of respiratory infections among these animals is well known; and that they succumb readily to the pneumococcus is evidenced by the fact that an epidemic of Pneumococcus Type IV pneumonia broke out among our stock monkeys and killed between 30 and 40 of them in less than 4 weeks. The disease ran through these animals, fresh from the Tropics, in very much the same manner that measles and pneumonia ravaged our southern recruits in 1917 and 1918. In either instance it was a case where an organism was suddenly brought in contact with a disease to which it had not been previously exposed. These monkeys when living in their natural environment probably rarely encountered the pneumococcus and had acquired no racial immunity to pneumococcus infections. Man, on the other hand, at least in North America, and particularly in urban communities, is constantly exposed to pneumococcus infections, and by reason of this exposure has probably gradually built up a fair degree of immunity against the microorganism. Clough[12] has recently shown that 19 per cent of normal men have demonstrable protective substances against pneu-

[12] Clough, P. W., *J. Am. Med. Assn.*, 1919, lxxiii, 785.

mococcus in their serum. Lack of previous exposure to pneumococcus is evidenced in monkeys by the difficulty which one has in producing protective substances in their blood.

A close analogy exists in this connection between pneumonia and typhoid fever. Metchnikoff and Besredka[13] in their study of experimental typhoid fever found that it was impossible to protect apes against the disease by means of killed cultures. Most of the typhoid vaccine, however, used in this country and elsewhere has been composed of killed bacilli and the results obtained with this vaccine are sufficient justification for its further use. To reason too closely, therefore, from monkey to man may lead to false conclusions. The bearing of this discussion on the question of prophylactic vaccination against pneumonia in man is obvious. The value of such vaccination will have to be finally determined by vaccinating large groups of men living under approximately the same conditions, and the results controlled by observations upon similar unvaccinated groups.

Pneumococcus vaccine probably stimulates in every case the production of a certain quantity of antibody, an amount, however, which in monkeys is not sufficient to protect them against pneumonia. Usually the antibody production in monkeys is not of sufficient degree to be demonstrable by any laboratory test. It is sufficient, however, to modify the course of the disease. The bacteremia is distinctly less marked. In twelve vaccinated monkeys the mortality rate was 41.6 per cent, while for seventeen unvaccinated monkeys the mortality rate was 76.4 per cent. Other evidence for this antibody production is furnished by the resistance which vaccinated monkeys offer to infection by the intravenous route.

In conclusion, it must be emphasized that immunity is a purely relative term. Almost any animal's "immunity," so called, can be overcome by a sufficiently large injection of virulent bacteria.

[13] Metchnikoff, E., and Besredka, A., *Ann. Inst. Pasteur*, 1911, xxv. 931; 1913, xxvii, 597.

CONCLUSIONS.

1. The subcutaneous inoculation of monkeys with Pneumococcus Type I vaccine in doses comparable with those employed in man does not protect them against subsequent attacks of Pneumococcus Type I pneumonia, either spontaneous or experimental. Furthermore, the occurrence of Pneumococcus Type IV pneumonia among monkeys that have been vaccinated with Pneumococcus Type I lipovaccine indicates that the vaccinated animals develop no cross-protection against other types of pneumonia.

2. Vaccination does, however, modify the course of the disease. Invasion of the blood stream by the pneumococcus in vaccinated animals is usually slight, and the proportion of recoveries is considerably higher for vaccinated than for unvaccinated monkeys.

3. Pneumococcus saline vaccine produces a greater amount of protective substance in the serum of the vaccinated animal than does pneumococcus lipovaccine and is probably, therefore, a better antigen. Both, however, fail to protect the animal against pneumococcus pneumonia.

4. Subcutaneous vaccination with pneumococcus vaccine gives definite protection against experimental pneumococcus septicemia. In other words, vaccination may induce a humoral immunity without protecting against intratracheal infection.

5. In view of the fact that monkeys are highly susceptible to pneumococcus infection, a strict analogy cannot be drawn between pneumococcus immunity in monkeys and pneumococcus immunity in man, since in the latter a considerable amount of resistance already exists, probably by reason of repeated exposure to pneumococcus infection.

EXPERIMENTAL STUDIES ON DIABETES.

Series I. Production and Control of Diabetes in the Dog. 3. Effects of Protein Diets.

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Comparison of Carbohydrate and Protein.

In mildly diabetic dogs the starch of 50 to 100 gm. of bread or cereal is more active in producing glycosuria than the larger quantity of potential carbohydrate represented in a kilo of beef lung.[1] Undoubtedly the starch would be much better assimilated if distributed in small doses over a longer time, but the supposition that the difference is only one of rapidity of absorption and metabolism is opposed by the following facts: (1) The glycosuria from starch in these animals generally lasts over 6 or even 12 hours, while phlorizin and respiration experiments prove that protein requires no longer time for absorption and deamination. (2) The effect of carbohydrate is often cumulative. For example, Dog B2–89 was free from glycosuria on 1 kilo of beef lung daily. On June 25, 50 gm. of bread were substituted for 250 gm. of the lung. The urine remained negative till a glycosuria of 0.75 per cent appeared on June 30 and 0.71 per cent on July 1. Then on return to the diet of 1 kilo of lung, the urine remained negative up to the following test in August. On August 5, 25 gm. of bread were substituted for 150 gm. of lung. A trace of glycosuria appeared on August 10, and 0.18 per cent on August 14. On August 15, nothing was fed but 50 gm. of bread, and an excretion of 1.4 per cent sugar in 140 cc. of urine resulted. On resumption of the diet of 1 kilo of lung glycosuria remained absent up to the time of another experi-

[1] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 397–399.

ment on September 17. (3) Blood sugar analyses to be reported later show that protein causes little hyperglycemia in mild diabetes, but in severe diabetes it produces a blood sugar curve resembling that of carbohydrate in milder cases, not only in height but also in rapidity of rise, thus confirming the statement (1) above concerning time relations.[2] Also the maximal dextrose-nitrogen ratios in the severest diabetes are a familiar proof that the carbohydrate of protein is excreted quantitatively and is as incapable of assimilation as preformed carbohydrate.

These facts conform to clinical observations and justify the earlier treatment of diabetes, from the time of Rollo onward, inasmuch as the restriction of preformed carbohydrate is after all the foundation stone of dietotherapy, and protein restriction comes secondary in both time and importance.

This simple point also has relation to the question of whether diabetes is a deficiency of utilization of glucose primarily and specifically or of other foods as well. The progressive impairment of protein metabolism, from the stage where protein is apparently assimilated almost perfectly to the stage where it causes hyperglycemia and glycosuria almost identical with those from preformed carbohydrate, has an important bearing upon this question but does not in itself furnish a decisive answer.

Comparisons of Proteins.

Dog B2–29.—This dog, having severe diabetes and a very low tolerance, with a remnant of approximately $\frac{1}{15}$ of the pancreas, was used for feeding tests with approximately 10 gm. of protein in different forms (Table I).

Tests with different forms of protein in other animals gave similar results. Also Dog B2–25, possessing $\frac{1}{12}$–$\frac{1}{15}$ of the pancreas, was used for a more thorough test of pancreas feeding. The body weight was 11.3 kilos on July 4, 1914; 500 gm. of fresh raw beef pancreas were fed daily until July 18, then 600 gm. daily till July 28, then 750 gm. of pancreas till Aug. 2, when the body weight was 14.4 kilos. Slight glycosuria was present from July 30 to Aug. 2. Fasting from Aug. 2 to 10 reduced the body weight to 12.5 kilos. A diet of 500 gm. of beef lung was then given daily to Aug. 18, 600 gm. daily to Aug. 27, 750 gm. daily to Sept. 28,

[2] *Cf.* one example already reported, Allen F. M., *Am. J. Med. Sc.*, 1917, cliii, 362, Chart 8, Dog 386.

800 gm. daily to Oct. 10, 1,000 gm. daily to Oct. 17, 1,200 gm. daily to Oct. 21, and 1,400 gm. daily to Nov. 28. Up to Oct. 29, the lung was boiled after weighing; after that date it was fed raw. Glycosuria was absent till Nov. 25, when 4.1 gm. of sugar were excreted, increasing to 8.2 gm. on Nov. 27. The body weight at this time was 17 kilos. The reason for the higher assimilation of beef lung at a higher body weight was that the dog was gaining tolerance with time. Evidently there is nothing in raw pancreas which alters the natural customary progress of this improvement.

TABLE I.

Dog B2–29.

Date.	Diet.	Glycosuria.
1913		*gm.*
Dec. 29	50 gm. of raw beef.	0
" 31	30 " " soy beans (boiled).	0.2
1914		
Jan. 1	70 " " egg (approximately 2 small eggs), raw.	Trace.
" 2	100 " " lard (no protein).	0
" 3	50 " " raw pancreas.	0.16

Several experiments for comparison of raw and cooked meat (ordinary beef for boiling or beef lung) are typified by the following example.

Dog D4–74.—Female; mongrel; age 4 years; moderately well nourished. Jan. 17, 1917. Partial pancreatectomy leaving remnant estimated at $\frac{1}{17}$. Weight throughout feeding experiments 13.2 to 13.5 kilos. The quantities of beef lung mentioned (Table II) were either boiled after weighing or fed raw.

The literature to which these experiments are related has been reviewed previously.[3] It was confirmed in numerous observations[4] that after a period of carbohydrate-free diet or especially of fasting the feeding of carbohydrate causes glycosuria, which ceases on continuance of the same carbohydrate diet. Apparently some state of unpreparedness of the body for the sudden flood of carbohydrate is

[3] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913, 442, 531, 813–815.

[4] *Cf.* Dog B2–63, Mar. 30 and Dec. 16, 1915 (Allen,[1] p. 387); also Paper 4 of this series (*J. Exp. Med.*, 1920, xxxi, 576).

TABLE II.

Dog D4–74.

Date.	Diet.	Glycosuria.
1917		*gm.*
Apr. 16	400 gm. of cooked lung; 100 gm. of suet.	0
" 17	500 " " " " 100 " " "	0
" 18	600 " " " " 100 " " "	0
" 19	600 " " " "	0
" 20	600 " " raw "	0
" 21	700 " " " "	0
" 22	700 " " cooked "	0
" 23	800 " " " "	0
" 24	800 " " " "	0.8
" 25	800 " " raw "	Faint.
" 26	800 " " cooked "	"
" 27	800 " " raw "	0
" 28	900 " " cooked "	Very faint.
" 29	900 " " raw "	0.25
" 30	900 " " cooked "	Faint.
May 1	1,000 " " raw "	Slight.
" 2	1,000 " " cooked "	Faint.
" 3	1,000 " " " "	Very faint.
" 4	1,000 " " " " 25 gm. of bread.	10.9
" 5	1,000 " " raw " 25 " " "	6.7
" 6	1,000 " " " "	5.1
" 7	1,000 " " " "	Slight.
" 8	1,000 " " cooked "	0.5
" 9	1,000 " " raw "	2.2
" 10	1,000 " " cooked "	Faint.
" 11	1,000 " " raw "	1.9
" 12	1,000 " " cooked "	Faint.
" 13	1,000 " " raw "	"
" 14	1,000 " " " "	0
" 15	1,000 " " cooked " 25 gm. of bread.	Faint.
" 16	1,000 " " raw " 25 " " "	3.7
" 17	1,000 " " " "	Faint.
" 18	1,000 " " cooked " 25 gm. of bread.	3.6
" 19	1,000 " " raw " 25 " " "	6.3
" 20	1,000 " " cooked " 25 " " "	0
" 21	1,000 " " raw " 25 " " "	5.7
" 22	1,000 " " cooked " 25 " " "	8.2
" 23	1,000 " " raw " 25 " " "	2.9
" 24	1,000 " " cooked " 25 " " "	3.4
" 25	1,000 " " raw " 25 " " "	4.1
" 26	1,000 " " cooked " 25 " " "	0
" 27	1,000 " " raw " 25 " " "	2.7
" 28	1,000 " " cooked " 25 " " "	2.2
" 29	1,000 " " raw " 25 " " "	1.3
" 30	1,000 " " cooked " 25 " " "	Very faint.
" 31	1,000 " " raw " 25 " " "	Faint.

here represented, just as later authors[5] have proved that a dose of sugar somehow prepares the normal organism so that a second dose is more perfectly assimilated.

Parenteral injection of pancreas extract may lower the sugar in blood or urine, like various other causes of intoxication or prostration, but no therapeutic benefit has ever been found from such treatment.[6] The feeding of pancreas has never benefited diabetes, either in human patients[7] or in dogs. Dogs are of value for these tests because they can eat much more in proportion to the body weight than human patients, but pancreas in either large or small quantity is found to produce glycosuria as readily as any other form of protein. Sandmeyer's observation that, when pancreatic juice is lacking, the improvement of digestion resulting from pancreas feeding may markedly increase glycosuria has been confirmed by Homans,[8] and serves further to discredit the therapeutic usefulness of pancreas or pancreatic preparations given by mouth. Reach's claim that any raw meat may have the same glycosuric influence as pancreas, through some "toxic" action, appears confusing. The necessity of close personal supervision of feeding experiments by the investigator may again be mentioned. Some dogs have a strong repugnance for raw meat; others are ravenous for raw meat and will scarcely touch cooked meat. Fickleness of appetite and digestion is especially to be watched for in the Sandmeyer type of diabetes. The most plausible assumption to explain Reach's results is that his dogs either refused or vomited the cooked meat. The above experiments prove that there is no appreciable difference between cooked and raw meat in regard to either the glycosuria resulting directly from them or their influence on the assimilation of carbohydrate.

[5] Hamman, L., and Hirschman, I. I., *Bull. Johns Hopkins Hosp.*, 1919, xxx, 306.

[6] Allen,[3] pp. 813–819, 855–857. Kleiner, I. S., and Meltzer, S. J., *Proc. Nat. Acad. Sc.*, 1915, i, 338.

[7] Allen, F. M., Stillman, E., and Fitz, R., Total dietary regulation in the treatment of diabetes, Monograph of The Rockefeller Institute for Medical Research, No. 11, New York, 1919, Chapter IV.

[8] Homans, J., *J. Med. Research*, 1915, xxxiii, 1.

Several experiments failed to show any sign of the wide glycosuric differences between proteins alleged by some authors in connection with the oatmeal "cure." There might be some possible interest in exact determinations of blood and urine sugar following the feeding of proteins differing in their content of sugar-forming and non-sugar-forming amino-acids, but the difficulties and uncertainties mentioned with regard to carbohydrate tests are still greater here. The above orientation experiments sufficed to exclude any differences of therapeutic importance.

Results of Immediate Protein Excess.

Dog B2–56.—Female; mongrel; white with brown patch over left eye; age 5 years; good condition; weight 16.5 kilos. Apr. 29, 1914. Removal of pancreatic tissue weighing 24.1 gm. Remnant about main duct estimated at 4.2 gm. (⅛). There was glycosuria for 3 days without feeding, probably because dissection about the pancreas remnant was followed by inflammation in it. Subsequently there was heavy continuous glycosuria on meat diet. May 14. Two tiny bits of tissue, weighing together only a small fraction of a gram, were removed from the pancreas remnant for examination. The remnant at this time did not appear on gross inspection as inflamed. Fasting was then imposed, May 14 to 23, but glycosuria was not reduced below 1.3 per cent and the diabetes was obviously uncontrollable. Meat was then given *ad libitum*, with resulting increase followed by decline of glycosuria and further loss of strength. May 28. The dog weighed 9.7 kilos, was too weak to stand, and was killed for autopsy.

Autopsy.—The pancreas remnant weighed 5.4 gm. and seemed normal in appearance and consistency. The gross autopsy was otherwise negative except for a large abscess in the right axilla, probably derived from a perforating ulcer at the elbow.

Microscopic Examination.—In the tissue removed on May 14 inflammation was limited almost entirely to broad bands of edematous fibrous tissue between lobules, infiltrated with leucocytes and also frequently hemorrhagic, while inside the lobules themselves there was little disturbance. The acini were normal and the islands markedly vacuolated. At autopsy the viscera, including the pancreas, seemed practically normal microscopically. Only slight thickening of trabeculæ remained from the previous infiltration. Acini were normal; islands more extensively vacuolated than before and reduced in size and number.

The diabetes was evidently made more severe by inflammation, but even under these circumstances glycosuria was reduced to traces and was on the point of disappearing with 3 days of fasting following

the first operation. By care in diet at this time control of the diabetes could presumably have been achieved, and after subsidence of the inflammation the recovery of a high tolerance could have been expected. Excessive protein diet removed this possibility and, in conjunction with the inflammation, caused a much more rapid course of diabetes and cachexia than usual.

Results of Prolonged Protein or Protein-Fat Diets.

Examples were previously given[9] of the existence of an apparent limit of protein tolerance in animals with the proper degree of diabetes; and when such a limit is exceeded, downward progress is natural and inevitable. As part of the protein molecule still remains available for nutrition, the decline of weight and strength is generally slower than on high carbohydrate diets, and life generally continues for several months, as illustrated in numerous former experiments. The above record of Dog B2–56 illustrates downward progress on protein as rapid as is usually seen on carbohydrate-rich diets, and also the facts that differences in the course of experimental diabetes are as marked as in clinical diabetes, and that the characteristic changes in the islands are not dependent on preformed carbohydrate in the diet. A less simple problem of protein feeding is presented under the following conditions.

The question arises whether, when an animal has diabetes, as demonstrated by glycosuria on carbohydrate feeding, but is able to eat protein to satiety without glycosuria, or when the protein ration is kept below the apparent tolerance and the full caloric requirements are supplied by addition of fat, diabetes is permanently avoided or merely delayed. It is essential that the test animals should have no changes in the pancreas tending to lower tolerance, and also that the actual permanency of the latent diabetes should be established and a tendency to spontaneous recovery excluded. With these precautions, it may be assumed that animals with simple resection of a portion of the pancreas are free from constitutional or other inherently progressive processes such as may be imagined in human patients. Tests with prolonged protein feeding in them are of the

[9] Allen,[3] p. 588, Dog 38, p. 777, Dog 154.

highest importance, to decide whether (notwithstanding the high content of potential carbohydrate in protein) the difference between protein and carbohydrate diet is absolute, or whether it is a difference of the kind above mentioned between sugar and starch, in that glycosuria is brought on merely more slowly but just as surely. Even with strict quantitative limitation of protein, the question may be stated on a broader basis with regard to the possibility of an impairment affecting the total metabolism in diabetes; namely, whether a diabetic organism can live out its full normal term of life at a full normal level of weight and metabolism by simple limitation of carbohydrate (preformed or from protein) in the diet, or whether the burden of general metabolism will suffice to wear out the weakened function so that an ultimate outbreak of frank diabetic symptoms will result. Such tests upon suitably chosen animals will contribute much toward the question of spontaneous downward progress in human diabetes and the efficacy of the classical treatment based on the idea of restriction of carbohydrate alone. Dogs are specially suited for such experiments, because of their relative insusceptibility to acidosis and other disturbances on pure protein or protein-fat diets.

The first dogs received upon beginning the investigation were set apart for these prolonged experiments. It was inevitable that deaths from distemper, rabies, and other accidents should spoil years of work in some cases, but by starting with a sufficient series of animals and substituting others as needed, some instructive long observations were obtained, of which the following four are the best examples.

Dog B2–00.[10]—Female; mongrel with some bull-terrier blood; brindle; age 3 years; good condition; weight 14 kilos. This dog was received on Oct. 25, 1913, and was subjected to five operations for removal of successive fractions of the pancreas, with periods of many months between so as to allow for any possible compensation by hypertrophy or alteration of assimilation from any cause, and with repeated tests of the carbohydrate tolerance at all stages. Tolerance was maintained for bread and soup with as much as 200 gm. of glucose up to Dec. 16, 1916, when the removal of only 0.1 gm. of pancreatic tissue brought on diabetes, so that bread and soup feeding alone sufficed for slight glycosuria. The dog

[10] See photograph at end of Paper 5 (*J. Exp. Med.*, 1920, xxxi, 587).

passed through two pregnancies in quick succession, the second one terminating on July 16, 1917. The observations up to this point will be described in detail later. Throughout this time she was given 1 kilo of beef lung daily, but was not required to eat all of it; glycosuria was absent except in occasional tolerance tests.

The freedom from glycosuria continuing, the following plasma sugar tests were performed.[11] The time of feeding was between 9 and 10 a.m. daily. July 26. Body weight 13.2 kilos. Dog unwell. Plasma sugar before feeding 0.055 per cent; 2 p.m., 0.099 per cent; 5 p.m., 0.122 per cent; 8.30 p.m., 0.081 per cent. Nov. 27. Weight 14 kilos. 2 p.m. Plasma sugar 0.123 per cent. Dec. 3. Weight 14.5 kilos. Plasma sugar before feeding 0.067 per cent; 5 p.m., 0.108 per cent. Dec. 27. Weight 12.2 kilos. Plasma sugar before feeding 0.128 per cent; 2 p.m., 0.151 per cent; 5 p.m., 0.128 per cent.

TABLE III.

Dog B2–00.

Time.	Aug. 9, 1917.		Oct. 5, 1917.		Nov. 22, 1917.		Dec. 18, 1917.	
	Plasma sugar.	Urine sugar.	Plasma sugar.	Urine sugar.	Plasma sugar.	Urine sugar.	Plasma sugar.	Urine sugar.
	per cent	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*
Before feeding....	0.081	0	0.109	0	0.109	0	0.141	0
2 hrs. after "	0.164	Very faint.	0.159	0	0.204	Faint.	0.400	3.55
4 " " "	0.109	Faint.	0.145	Faint.	0.238	0.14	0.384	2.10
6 " " "	0.135	"	0.152	0.14	0.125	0.11	0.357	3.95

Four tolerance tests also indicated a decline of tolerance during the above period of high protein diet. These tests consisted in giving, on the 4 days mentioned, an identical test diet of 200 gm. of bread, 150 gm. of glucose, and 100 gm. of beef lung, and determining the blood and urine sugars at the intervals shown in Table III.

Jan. 2, 1918. Diet changed to 400 gm. of lung and 50 gm. of suet. Jan. 15. Weight 12 kilos. Plasma sugar before feeding 0.139 per cent; 5 p.m., 0.161 per cent. Jan. 17. Diet changed to bones only. Jan. 21, 2 p.m. Plasma sugar 0.164 per cent. Jan. 28. Weight 11.1 kilos. Plasma sugar before feeding 0.133 per cent. Diet changed to 100 gm. of lung and 100 gm. of suet. Feb. 6, 2 p.m. Plasma sugar 0.155 per cent. Feb. 11. Plasma sugar before feeding 0.112 per cent; 2 p.m., 0.123 per cent. Feb. 14. Diet increased to 300 gm. of lung and 100 gm. of suet. Mar. 14. Diet increased to 500 gm. of lung and 100 gm. of suet.

[11] The methods of Lewis and Benedict (Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, xx, 61), and of Benedict (Benedict, S. R., *J. Biol. Chem.*, 1918, xxxiv, 203) have been used for all blood sugar analyses.

On protein diet from July 26, 1917, to January 15, 1918, the dog showed downward progress as evidenced by the increasing hyperglycemia and the tolerance tests. To exclude the possibility that such a hyperglycemic tendency might be due to something inherent in the dog, undernutrition was instituted on January 17 with a diet of nothing but fresh bones, and thereafter the quantity of protein was restricted, the maximum of 500 gm. of lung being attained by March 14. Hyperglycemia which is slow in onset, due to prolonged excess in protein or fat, is also slow in subsiding; but with the decline in body weight the blood sugar came gradually to the normal level. The last mentioned diet and the freedom from glycosuria continued to August, 1918.

Dog B2–01.[10]—Female; bull and fox-terrier mongrel; white with brown markings; age 2 years; good condition; weight 14 kilos. Received Oct. 25, 1913, and used at first like Dog B2–00 for removal of successive portions of pancreas.[12] The removal of 0.8 gm. of tissue in the final operation on Aug. 31, 1916, made the animal potentially diabetic.[13] The tolerance was at first so high that bread diet with 300 gm. of glucose caused no glycosuria. It declined till glycosuria resulted from bread alone, then fluctuated according to the body weight, but on the whole fell in consequence of prolonged slight overfeeding, so that by June 30, 1917, there was hyperglycemia when the dog was fat on a diet of 400 gm. of lung and 200 gm. of suet. The weight was kept low and the tolerance high during the remainder of that year. In 1918 the dog was allowed to gain weight, on a diet which after Feb. 13 was 400 gm. of lung and 100 gm. of suet. The plasma sugar was normal (0.089 per cent) before feeding in the last analysis on Feb. 4.

Dog D4–52.[10]—Female; mongrel; yellow and white; age 3 years; good condition; weight 12 kilos. Nov. 24, 1916. Received with pups, which she reared. June 27, 1917. Removal of pancreatic tissue weighing 22.9 gm.; remnant about main duct estimated at 3.4 gm. ($\frac{1}{8}$). Glycosuria at first was heavy on feeding of bread and soup with glucose up to 200 gm., but soon ceased. July 12. 0.35 gm. of additional pancreatic tissue was removed, and the same experience with feeding was repeated. July 20. 0.2 gm. of additional pancreatic tissue was removed, and again the glycosuria from bread and 200 gm. of glucose was transitory. Aug. 3. A final fragment of 0.3 gm. of pancreatic tissue was removed. Glycosuria was absent on bread diet until Aug. 8, when the addition of 200 gm. of glucose made it heavy. On this glucose mixture the sugar excretion gradually diminished and ceased on Aug. 13.

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[12] These experiments will be described later.

[13] The subsequent feeding tests will be described later.

After Aug. 21, glucose was omitted and the diet was plain bread and soup. Slight glycosuria appeared on Oct. 10, and the diet was therefore changed to 500 gm. of beef lung and 100 gm. of suet. The same diet was continued regularly, though frequently the dog left considerable of it uneaten. By June, 1918, the weight had risen to a maximum of 12.6 kilos. Tests were performed at intervals (Table IV).

TABLE IV.

Dog D4–52.

Feeding of 500 gm. of lung, 100 gm. of suet, and 100 gm. of bread.

Date.	Weight.	Plasma sugar.			Urine volume.	Glycosuria.
		Before feeding.	3 hrs. after feeding.	6 hrs. after feeding.		
1917	*kg.*	*per cent*	*per cent*	*per cent*	*cc.*	*per cent*
Mar. 1	11.7	0.110	0.122	0.109	420	0
Oct. 25	11.6				580	2.44
Nov. 14	11.7	0.092	0.294	0.246	410	1.1

TABLE V.

Dog D4–52.

Feeding of 300 gm. of bread and 200 gm. of glucose.

Date.	Weight.	Plasma sugar.				Glycosuria (24 hrs.).
		Before feeding.	2½ hrs. after feeding.	4 hrs. after feeding.	6½ hrs. after feeding.	
1917	*kg.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*
Aug. 8	11.5	0.149		0.312	0.092	6.75
" 15	11.6	0.101		0.172	0.161	0
Oct. 5	11.5	0.208	0.334	0.384	0.416	18.0

The tests mentioned above merely compare the diabetic condition on October 25 and November 14 with the normal state on the preceding March 1. As far as can be judged from glycosuria, there was gain rather than loss of tolerance from October 25 to November 14, though such differences may be accidental.

From the tests in Table V, it is seen that on August 8 (5 days after the last pancreas operation) the above glucose mixture caused hyperglycemia and glycosuria, which were brief, both being ended within the $6\frac{1}{2}$ hour experimental period. By August 15, the bread and glucose

diet having been continued in the interval, tolerance had apparently been gained, so that glycosuria no longer resulted; but the curve of hyperglycemia, though lower, was longer. Delayed absorption is one possible factor here. The dog tired of glucose before diabetes was produced, so that after August it had to be discontinued, as already stated. On plain bread diet thereafter glycosuria was absent, but hyperglycemia was evidently present and tolerance was lost markedly. This was shown in the tolerance test of October 5, in which the blood sugar was high at the outset and ran a prolonged high course. Of the 18 gm. output, only 7.9 gm. were excreted during the $6\frac{1}{2}$ hour period, and the rest over night. A fast day was given on October 6, to allow recovery from the test. Nevertheless, glycosuria on plain bread and soup feeding began on October 10 as stated. This experiment is another illustration of downward progress on starch diet, and also of downward progress with hyperglycemia without glycosuria. A recuperative effort on the part of the pancreas remnant is manifest, but it was overcome by the excessive feeding.

Intravenous Glucose Tests.

The intravenous injections were given discontinuously, in a manner described in detail in a later paper. The dosage was constant, on the assumed normal weight of 12 kilos, without regard to the changes in actual weight. This meant the injection of 30 cc. of 10 per cent glucose solution every 15 minutes, in order to give 1 gm. per kilo per hour (Table VI). Feeding was omitted on each injection day and the day following. Excitement and other known causes of disturbance were avoided.

The experiment on August 6, 3 days after the last pancreas operation, seems to show the same characteristics as the above described feeding test on August 8; namely, beginning with an existing hyperglycemia, the blood and urine sugars rose quickly to high levels, but tended to fall toward the close, the relatively low percentages of the 7th and 8th hours being particularly striking.

The observations of November 19 and February 19 show the existence of hyperglycemia on prolonged carbohydrate-free diet, and downward progress of the diabetes notwithstanding absence of glycosuria.

TABLE VI.

Dog D4–52.

Intravenous glucose injections, 1 gm. per kilo per hour in 10 per cent solution (four injections per hour).

Time.	Plasma sugar.	Urine.	
		Volume.	Glucose.
Aug. 6, 1917. Weight 10.75 kilos.			
	per cent	*cc.*	*per cent*
Before injection	0.218		0
At end of 1st hr.	0.500	16	2.64
" " " 2nd "	0.715	16	5.08
" " " 3rd "	0.475	54	2.84
" " " 4th "	0.590	52	1.54
" " " 5th "	0.415	76	0.74
" " " 6th "	0.270	78	1.81
" " " 7th "	0.202	94	1.45
" " " 8th "	0.125	82	0.82
1 hr. after injection	0.128	53	0.19
2 hrs. " "	0.130	12	Faint.
3 " " "	0.133	29	0
Glucose excreted			7.8 gm.
Nov. 19, 1917. Weight 11.7 kilos.			
Before injection	0.169		0
At end of 1st hr.	0.555	36	1.93
" " " 2nd "	0.555	68	3.08
" " " 3rd "	0.435	82	1.49
" " " 4th "	0.370	132	0.79
" " " 5th "	0.370	98	0.39
" " " 6th "	0.356	126	0.28
" " " 7th "	0.370	127	0.55
" " " 8th "	0.370	105	0.39
" " " 9th "	0.384	105	0.36
" " " 10th "	0.322	101	0.42
1 hr. after injection	0.156	45	Faint.
2 hrs. " "	0.147	10	0
Glucose excreted			7.7 gm.
Feb. 19, 1918. Weight 12 kilos.			
Before injection	0.145		0
At end of 1st hr.	0.417	20	4.77
" " " 2nd "	0.476	55	5.13
" " " 3rd "	0.500	114	3.39
" " " 4th "	0.525	109	3.45
" " " 5th "	0.500	102	3.40
" " " 6th "	0.475	140	2.78
" " " 7th "	0.455	90	4.35
" " " 8th "	0.384	87	3.23
1 hr. after injection	0.294	43	0.74
2 hrs. " "	0.170	19	Very faint.
Glucose excreted			25.7 gm.

Dog D4–69.—Male; black collie mongrel; age 3 years; good condition; weight 15.5 kilos. Jan. 5, 1917. Received. The removal of a kidney on this date was presumably without effect on the production of diabetes. Successive portions of pancreatic tissue were removed on Jan. 23, Feb. 23, Mar. 28, Apr. 19, May 8, June 1, June 27, and July 20. Tests of the tolerance were made between all the above operations, and diets of bread and soup with as high as 400 gm. of glucose, though causing more and more glycosuria, failed to maintain it permanently. After the last operation, which involved the removal of only 0.5 gm. of tissue, glycosuria was absent on bread and soup diet, but heavy at first with the addition of 100 gm. of glucose. As it tended to diminish, the glucose was increased on Aug. 8 to 200 gm., but again the heavy glycosuria diminished, became intermittent, and ceased. No glycosuria occurred after Aug. 15. Aug. 21. Glucose

TABLE VII.

Dog D4–69.

Feeding of bread and soup and 200 gm. of glucose.

Date.	Plasma sugar.			Glycosuria (24 hrs.).
	Before feeding.	3½ hrs. after feeding.	5½ hrs. after feeding.	
1917	*per cent*	*per cent*	*per cent*	*gm.*
Aug. 8	0.133	0.344	0.310	4.5
" 9	0.116	0.238	0.264	Trace.
" 15	0.110	0.217	0.212	0
Oct. 5	0.200	0.525	0.435	24.8

was discontinued, and the diet thereafter was plain bread and soup. The weight had risen by Oct. 5 to 17.6 kilos. Tolerance tests were performed during this period (Table VII).

Though 2 fast days were imposed following Oct. 5, the former sugar freedom on bread diet was no longer possible, the glycosuria in repeated attempts being persistent and increasing. Therefore, beginning Oct. 17, the diet was 100 gm. of suet and quantities of lung varying from 400 to 1,000 gm. Oct. 25 to 30. A diet of 500 gm. of lung and 100 to 150 gm. of bread caused continuous glycosuria. After this the diet was 500 gm. of lung and 100 gm. of suet, with continuous absence of glycosuria except on a few test days.

Nov. 14. Addition of 100 gm. of bread to the diet caused glycosuria of 0.3 per cent in 350 cc. of 24 hour urine. The plasma sugar before feeding was 0.127 per cent; 3 hours after feeding, 0.244 per cent; 6 hours after feeding, 0.138 per cent.

Intravenous tolerance tests were performed as shown in Table VIII.

Experiments like those on Dogs B2–00, B2–01, D4–52, and D4–69, with their requirements of prolonged care in diets, daily urinalyses, and attention to numerous details, are full of difficulties. These four dogs were the survivors of a series in which the attempt was made to reproduce the conditions of human patients as closely as possible. The above records extend to the summer of 1918, when the writer entered military service, and the dogs were left on the

TABLE VIII.

Dog D4–69.

Intravenous injection of 1.3 gm. per kilo per hour of a 10 per cent solution of glucose.

Time.	Aug. 6, 1917. (Weight 16.2 kilos.)			Dec. 4, 1917. (Weight 14.2 kilos.)			Feb. 19, 1918. (Weight 15.5 kilos.)		
	Plasma sugar.	Urine.		Plasma sugar.	Urine.		Plasma sugar.	Urine.	
		Volume.	Glucose.		Volume.	Glucose.		Volume.	Glucose.
	per cent	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*
Before injection.	0.106	10	0	0.104		0	0.154	32	0
After 1st hr.	0.278	20	1.56	0.715	100	2.84	0.625	46	4.83
" 2nd "	0.294	30	2.87	0.990	144	4.45	0.800	214	4.00
" 3rd "	0.312	64	1.71	0.990	222	3.65	1.000	252	4.35
" 4th "	0.270	106	0.76	1.000	300	2.80	1.180	274	3.70
" 5th "	0.218	110	0.42	0.844	220	2.93	1.110	281	4.25
" 6th "	0.204	76	0.53	0.844	265	2.43	1.050	270	4.55
" 7th "	0.183	128	0.51	0.625	222	2.71	1.000	222	4.55
" 8th "				0.715	216	3.82	0.950	278	5.13
1 hr. after last injection.	0.082	90	Trace.	0.370	60	3.60	0.525	78	5.88
2 hrs. " " "	0.098	82	0	0.217	66	0.49	0.322	10	5.13
Glucose excreted.....			4.5 gm.			55.2 gm.			80.4 gm.

Both the feeding and the intravenous tests indicated gradual loss of tolerance in the absence of glycosuria, first on starch and later on protein-fat diet. The sugar curves of Feb. 19, 1918, were not only the highest and most prolonged, but also with the initial figure of 0.154 per cent indicated that hyperglycemia was now constant on the regular diet of lung and suet. In the final blood taken at the height of digestion at 2.30 p.m. on Mar. 20, 1918, the plasma sugar was 0.256 per cent, still without glycosuria. The weight was gradually falling, and was 14.75 kilos at this time.

diets stated under the care of the animal attendants. They were seen once during the winter, sufficiently to learn that all four were free from glycosuria; three of them were vigorous and fat, but Dog D4–69 was thin and in rather poor condition. In the late summer of 1919, two of the dogs were found to have hyperglycemia and glycosuria, and were therefore transferred from The Rockefeller Institute to the writer's clinic. The two which were sugar-free were cared for at the farm of the Institute in New Jersey.

The animal in worst condition was Dog D4–69, which was thin, with hopeless hyperglycemia and glycosuria, and which died of diabetes on October 10, 1919. Dog B2–01 was strong and fat at a weight of 14.8 kilos, but showed heavy sugar and acetone reactions. Glycosuria was at first abolished by fasting, but returned, owing to persistence of hyperglycemia when attempts were made to feed. Rigorous undernutrition at this time might have proved successful, but the mistaken laxness, due to the deceptive strength and fatness of the animal, soon ended in a hopeless condition, which was uncontrollable by fasting, so that death occurred November 15, 1919. The record of the terminal period is given in the next paper. The other two dogs were in excellent condition when visited at the farm on November 3, 1919. Plasma sugar analyses on that day were as follows: Dog B2–00, before feeding, 0.092 per cent; 4 hours after feeding, 0.136 per cent; Dog D4–52, before feeding, 0.093 per cent; 4 hours after feeding, 0.123 per cent.

It will be seen that the experiment consisted in taking four dogs with different grades of diabetes, which had been produced by sufficiently long experimental procedures to rule out accidental influences as far as possible, and had been tested in various feeding and injection experiments, and then placing them on nearly identical diets on which they were free from glycosuria for extended periods. The ultimate outcome could in the main have been predicted from the preliminary observations.

Dog D4–69 had continuous hyperglycemia in the spring of 1918, and in the absence of extraneous interfering factors such a condition in dogs always leads to manifest and fatal diabetes. Loss of weight may have been due to the latent diabetes or to indigestion or other causes; it evidently postponed the outbreak of active dia-

betes longer than usual. Dog B2–01 exhibited hyperglycemia and lowered tolerance when obese in the summer of 1917, and it was therefore to be expected that a similar result would follow when she became fat on the same protein ration in 1918–19. These records illustrate that in dogs, as in human patients, a true recuperative power is limited to the earlier stages of diabetes, and when diabetes has lasted many months or years the assimilation may undergo apparent fluctuations according to the diet and weight but is not capable of any great restoration in an absolute sense. The final purpose was, after the expected onset of diabetes, to check this as before by fasting and reduced diet, and then prove that the animals could be kept indefinitely in the undernourished condition free from diabetes or downward progress, on the same protein ration; in other words, to show the influence of dietary fat and body weight in causing downward progress. This fact was established by other experiments,[12] but the opportunity of testing the permanency of such arrest of diabetes and the feasibility of protecting the assimilation by undernutrition extending over many years or the full lifetime of the dogs was lost.

Dogs B2–00 and D4–52 had milder diabetes. Both showed downward progress on excessive diets of carbohydrate or protein, and both illustrated the fact, often observed in human patients, that when mild diabetes is checked by a diet within the actual tolerance, hyperglycemia may persist for a long time but gradually subsides. The question was what would happen to such animals if they were allowed to live indefinitely on a diet which seemed to be within the tolerance. The result could not be predicted in advance, and the outcome to date establishes the following conclusions.

1. No inherent downward progress is perceptible. Dog B2–00 has been kept for 6 years, and has been demonstrably diabetic for 3 years. The downward progress observed in other animals, and also in these animals on excessive diets, is purely the result of food injury; in other words, to functional overstimulation of the pancreas as an endocrine organ.

2. The benefit of the classical treatment of diabetes is confirmed. With the susceptibility of both these animals to injury from excess of either carbohydrate or protein demonstrated, it is evident that

this injury was checked when carbohydrate was omitted, protein restricted, and a full caloric diet made up by the use of fat. Fat is evidently less injurious than carbohydrate or protein, and its harmfulness is chiefly noticeable in the more severe grades of diabetes. It is safer for these animals to be obese on a fat diet than to eat carbohydrate or a carbohydrate-forming food such as protein. By inference, fat is not a direct source of carbohydrate.

3. The future outcome in these two animals holds several possibilities. (*a*) One question concerns the degree and permanence of their assimilative power. Other experiments justify the assumption that by undernutrition their tolerance could be greatly raised, so that they might take considerable protein and carbohydrate, and by increased obesity the tolerance could be further lowered, probably to the point of glycosuria on their present protein ration. The question is whether their tolerance at their present weight is high enough for permanent assimilation of the limited carbohydrate derivable from their present diet; whether the difference between fat and protein is absolute or merely a matter of time, like the differences between glucose and starch or between starch and protein; and therefore whether diabetes is primarily a deficiency of the total metabolism or of carbohydrate metabolism alone. (*b*) The plasma sugars under present conditions are not absolutely normal. Particularly in Dog B2–00 the figure of 0.136 per cent during digestion of protein is above normal, but yet is within the limits permitted in numerous diabetic patients. There is a question whether this slight and temporary overload of each day can be borne indefinitely by the pancreas, or whether it is the first small sign of a breaking strain. (*c*) In addition to the simple prolonged functional wear and tear, other influences such as age enter in, and it is of interest to know whether senility will bring an onset of diabetes, as it seems to do in so many human patients. If necessary, the direction of progress in the animals can be judged at any time by tolerance tests, which were instituted in the first place in order to permit of such comparisons, but it seems better to avoid this source of possible injury. As these dogs bear such a close resemblance to mildly diabetic patients kept sugar-free by restriction only of sources of carbohydrate, it seems most valuable to learn whether they can remain fat and lazy indefinitely

with impunity, or whether even the mildest diabetes will ultimately undergo aggravation from luxus diets.[14]

CONCLUSIONS.

No specific differences were observed between the glycosuric effects of different kinds of protein. Other incidental observations were noted in connection with the records of individual animals. General conclusions are deferred to the close of the series.

[14] Both these dogs recently died and were autopsied. Dog D4-52 had a large calculus in the bladder but otherwise appeared to be in excellent condition. No cause of death was found. Dog B2-00 was more closely observed; there was a history of refusal of food, and drinking and vomiting of water. The urine in the bladder was heavy with sugar; the subcutaneous tissue and peritoneum contained abundant fat, and the liver was intensely fatty. It is therefore certain that the death of this animal was due to acidosis. The same possibility exists for Dog D4-52 but is unproved. The results indicate that luxus diets lead to a fatal termination in diabetes.

EXPERIMENTAL STUDIES ON DIABETES.

Series I. Production and Control of Diabetes in the Dog. 4. Control of Experimental Diabetes by Fasting and Total Dietary Restriction.

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In a previous publication the writer reported the results of attempts to produce a satisfactory experimental reproduction of clinical diabetes and a study of its nature, which was made to obtain some clue leading to its therapeutic control.[1] This investigation was carried to a point where it was believed, as stated in the preface, that the possession of the necessary working model of the disease had made the cure of diabetes a feasible laboratory problem. Out of numerous lines of therapeutic approach suggested from the literature, speculation, or experiment, two seemed worth following but had to be left unexplored. One of these consisted in attempts to stimulate or strengthen the pancreatic function in some direct manner, and it is still hoped to proceed in this direction at some later date. For certain reasons it was desirable to follow the other path first.

The phenomenon which suggested this latter research was the prevention or cessation of diabetes with ligation of the pancreatic duct.[2] It was at first suspected that this was due to improvement of the internal pancreatic function, through stoppage of the external secretory activity or even through structural changes involving increase of island tissue. When the work was finished, there was leisure for the following comparison with other observations. In Dog 55[3] diabetes ceased with the onset of distemper and the attendant loss of appetite and weight. In Dog 57, fasting for a week prior to operation seemed to prevent the onset of glycosuria. Likewise peritonitis and other infections often hindered the occurrence of glycosuria, though in some instances diabetes appeared as usual, notwithstanding the presence of peritonitis. Several other dogs[4] showed absence or cessation of glycosuria in consequence of illness, fasting, and loss of weight.

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[1] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913.

[2] Allen,[1] Chapter XXII.

[3] Allen,[1] p. 493.

[4] Allen,[1] pp. 771–772.

Also in an animal with fairly severe and advanced diabetes,[5] glycosuria was twice stopped by fasting, and downward progress in consequence of the overfeeding during the interval was demonstrated by the lower tolerance for protein at the close of the second as compared with the first fast. There are reports in the literature of the apparent cessation of diabetes with an intercurrent cachectic disorder,[6] and also of the benefit of the occasional therapeutic fast days introduced by Bouchardat, Cantani, and Naunyn. In addition, shortly before the beginning of the new research, Joslin emphasized the subsidence of diabetic symptoms in a severe case after the onset of tuberculosis with emaciation.[7] It thus seemed possible that the cessation of glycosuria from ligation of the pancreatic duct resulted from impaired food absorption and undernutrition. Homans[8] confirmed the phenomenon itself and demonstrated this as the true explanation by direct experiments.

The first opportunity was taken to try reduction of weight and total metabolism for the treatment of diabetes. The first dogs were chosen for the longest experiments, as explained in the preceding paper. (It so happened that Dog B2-05 furnished another illustration of the cessation of glycosuria with distemper.) The tests consisted in taking dogs, generally with much smaller pancreas remnants than those with which downward progress and death were found on full diets in the preceding paper, and determining to what extent active symptoms and impairment of assimilation could be prevented by fasting and total dietary restriction. Many examples are available and are mentioned incidentally in other papers, because the method was used as a routine for controlling diabetes. As the longest records are the most instructive for the present purpose, three experiments of 1 to $1\frac{3}{4}$ years in duration are here summarized as successful cases. Before proceeding to these, brief consideration may be given to the status of the so called "hunger glycosuria" in this connection.

Dog B2-52.—Female; bull-terrier mongrel; brindle; excellent condition; weight 12.8 kilos. Apr. 24, 1914. Removal of pancreatic tissue weighing 20.4 gm. Remnant about main duct estimated at 3.1 gm. (slightly over $\frac{1}{8}$). Glycosuria

[5] Allen,[1] Dog 64, pp. 354–361, 480.

[6] Allen,[1] p. 800 ff.

[7] Benedict, F. G., and Joslin, E. P., *Carnegie Inst. Washington, Pub. No. 136*, 1910. Joslin, E. P., Treatment of diabetes mellitus, Philadelphia and New York, 2nd edition, 1917, 409.

[8] Homans, J., *J. Med. Research*, 1915, xxxiii, 1.

was absent till bread and soup were fed on Apr. 30. It was maintained till May 7 by the addition of as much glucose as possible, but then ceased largely because of the dog's distaste for sugar. May 12 to 15. Fasting was imposed. The feeding of bread and soup with 50 to 75 gm. of glucose daily then caused glycosuria of 0.7 per cent on the 1st day, but none thereafter, and a second operation was necessary to make the dog diabetic.

This dog is representative of several tested in relation to hunger glycosuria, some of which were previously mentioned.[9] The point involved is the seeming conflict between the use of fasting and undernutrition for raising tolerance, and the experience of Hofmeister and others concerning the tendency to glycosuria created by starvation and malnutrition. When a dog on the border-line of diabetes refuses to eat enough to keep up glycosuria and break down tolerance, it might seem a promising plan to fast for a number of days and then feed bread and glucose, with the double idea that the glycosuric tendency would be increased by the fast, and also that the animal would eat more. Hofmeister's work is confirmed in these dogs much more strikingly than in normal dogs, and heavy glycosuria often occurs on feeding bread either alone or with sugar. The same phenomenon has frequently been witnessed in dogs changed suddenly from a protein or fat diet to a carbohydrate diet. But no matter how heavy the glycosuria, it is transitory just as in Hofmeister's normal dogs, and it has never been possible to produce diabetes thus in any animal which was non-diabetic on the same diet before the fast. The phenomenon seems to illustrate some state of unpreparedness of the body for the unaccustomed carbohydrate flood, but it does not represent any true diabetic tendency or any exception to the general rule that the pancreatic function is strengthened by fasting.

Dog B2-25.[10]—Male; mongrel; tall, rough haired, brown and white; age 3 years; moderately well nourished; weight 18.2 kilos. Dec. 16, 1913. Removal of pancreatic tissue weighing 30.1 gm. Remnant about main duct estimated at 2.6 gm. ($\frac{1}{12}$–$\frac{1}{8}$). The further record is contained in Table I. Dec. 3, 1914. In excellent health and strength; accidental death.

Autopsy.—Negative grossly and microscopically. Pancreas remnant, fully normal in appearance and consistency, weighed 8.25 gm. Microscopically, acini normal; islands small and scarce, free from vacuolation.

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[9] Allen,[1] pp. 586–587. *Cf.* Paper 3, p. 557; also Dog B2-51, p. 578.

[10] See photograph at end of Paper 5 (*J. Exp. Med.*, 1920, xxxi, 587).

The animal lived approximately a year, was most of the time hungry and excessively thin, but vigorous and lively, in contrast to the weakness of diabetes. Tolerance was very low at the outset, corresponding to the small pancreas remnant; but instead of downward progress there was a very great gain in assimilation. The clinical absence of diabetes was corroborated by the intact state of the islands.

TABLE I.

Dog B2–25.

Date.	Body weight.	Diet.	Urine.	
			Volume.	Glucose.
1913	*kg.*		*cc.*	*per cent*
Dec. 17	18	Fasting.	1,025	0
" 18		200 gm. of meat.	1,500	1.0
" 19		Fasting.	1,700	0.6
" 20		"	875	Faint.
" 21–26		"		0
To May, 1914	Down to 11.	From 30 gm. of lung and 30 gm. of suet to 120 gm. of lung and 120 gm. of suet.		Traces of glycosuria at first on the higher diets; later none.
" Dec., 1914	Increase to 17.	Pancreas or lung increasing as described in preceding paper,* up to 1,400 gm. daily.		Glycosuria absent till Nov. 25–27; then 4.1 to 8.2 gm.; stopped by a fast day.

* Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 556.

Dog B2-51.—Male; mongrel; yellow and white; age 1½ years; moderately well nourished; weight 9.75 kilos. Mar. 31, 1914. Removal of pancreatic tissue weighing 20.1 gm. Remnant about main duct estimated at 2.8 gm.(⅛). Beginning Apr. 2, a diet of 200 cc. of milk daily caused glycosuria as high as 2.6 per cent, and on Apr. 5 the change to bread and soup diet resulted in glycosuria of 5 per cent; but this gradually subsided and could only be maintained by the addition of 50 gm. of glucose, which was as much as the dog would take on account of a decided distaste for sugar. With increasing indigestion and loss of weight, glycosuria ceased on Apr. 20.

Apr. 21. Body weight 8 kilos; opening the abdomen revealed obvious marked hypertrophy of the pancreas remnant, from which 1.5 gm. of tissue were removed. Glycosuria was then absent on meat diet. Apr. '26. An abrupt change to bread and soup produced glycosuria as usual for 1 day; it then remained absent even with the addition of 50 gm. of glucose on Apr. 30.

May 1. An additional 1.8 gm. of pancreatic tissue was removed, the body weight being 8.1 kilos. A slight glycosuria ensued on that day, then was absent on beef lung, and after May 7 on bread and soup with 50 gm. of glucose. The emaciated and cachectic condition was a sufficient explanation; when the full diet was forced it was probably largely lost through diarrhea, and on May 12 the weight was down to 6.4 kilos. Accordingly meat diet *ad libitum* was then resumed, with gradual

TABLE II.

Dog B2–51.

Date.	Body weight.	Diet.	Glycosuria.
1914	*kg.*		*per cent*
July 1–9	8.7–7.6	100 gm. of lung.	0
" 10–22	7.6–7.0	200 " " "	0
" 23–30	6.9–6.75	250 " " "	0
" 31–Aug. 9	6.7–6.6	300 " " "	0
Aug. 10–14	6.6–6.5	325 " " "	0
" 15–19	6.5–6.7	100 " " " 100 gm. of suet.	0
" 20–26	6.8–6.5	100 " " " 100 " " "	0
" 27–Oct. 6	6.6–7.2	100 " " " 75 " " "	0
Oct. 7–15	7.2	100 " " " 50 " " "	0
" 16–Nov. 5	7.2–6.8	100 " " " 30 " " "	0
Nov. 6–Dec. 1	6.9–7.2	100 " " " 30 " " " 50 gm. of lard.	0
Dec. 2–13	7.2	150 " " " 30 " " "	0
" 14–21	7.2–6.6	150 " " "	0
" 22–Jan. 3, 1915	6.5	150 " " " 50 " " lard.	0
1915			
Jan. 4–Feb. 17	6.6–8.0	150 " " " 100 " " "	0
Feb. 18–Mar. 1	8.0–8.1	200 " " " 50 " " "	0
Mar. 2–18	8.2–8.9	150 " " " 100 " " "	0
" 19	8.8	400 " " "	Trace.
" 20	8.8	400 " " "	0.16
" 21	8.6	Fasting.	0.36
" 22	8.4	100 gm. of lung.	0.25
" 23	8.4	100 " " "	0

gain in weight and health. By June 24, the weight had risen to 9.3 kilos. Glycosuria, which had remained absent before, suddenly appeared and continued for 5 days, the highest percentage being 1.2. It was stopped by 2 fast days; the diet and progress after these fast days are shown in Table II. Mar. 26, 1915. Accidental death.

Autopsy.—The pancreas remnant, normal in appearance and consistency, weighed 2.75 gm. Islands were rather few and small, but within normal limits.

Occasional cells in them showed slight vacuolation. Liver moderately fatty. Otherwise autopsy negative, including microscopic examination of thyroid, liver, kidneys, adrenals, and hypophysis.

Pancreatic tissue removed by operation Apr. 21, 1914, normal except for slight vacuolation in occasional island cells. Pancreatic tissue removed May 1, 1914, normal except for slight inflammatory reaction in places; no vacuolation in islands.

It is probable that the dog might have been made diabetic after the first operation if the sugar feeding could have been forced as in some other animals. This is suggested by the glycosuria and the anatomic evidence of overtaxed function in the island cells; but at the same time hypertrophy of the remnant and fall of body weight had the opposite influence, and repugnance to carbohydrate, indigestion, and diarrhea closed the attempt. Prior to the second operation, glycosuria was absent, and correspondingly the islands showed no vacuolation. Shortly preceding death there was slight glycosuria on protein diet, and this was paralleled as usual by slight hydropic changes in the islands. This experiment is one illustration that neither the size of the pancreas remnant, inflammatory changes, the length of time since a preceding operation, nor the character of the diet (carbohydrate or protein) govern this change in the islands, but only the presence or absence of active diabetes.

The record from May 1 to July 1, 1914, illustrates absence of diabetes in an animal in an emaciated condition on pure protein diet, and the loss of tolerance and outbreak of active symptoms when weight was gained on this diet.

The subsequent record shows the absence of diabetes on a restricted protein-fat diet, frequently adjusted in minor details to suit the dog's appetite and digestion, but so planned as to keep the body weight generally low. The animal was lively and fairly strong meanwhile. There was no tendency to real recovery, for when the weight was allowed to rise at the end, it was found that 400 gm. of lung sufficed to cause slight glycosuria. The slight vacuolation found in the islands resulted from such periods of overfeeding. The principal point of the experiment was the ability to keep a thin animal with diabetes of this degree of severity free from symptoms, without evidence of downward progress or indications that the condition could not have been continued indefinitely.

Dog D4-28.—Male; mongrel; yellow and white; age 2 years; good condition; weight 12 kilos. Sept. 28, 1916. Removal of pancreatic tissue weighing 25.3 gm. Remnant about main duct estimated at 2.3 gm. ($\frac{1}{12}$). As this dog had been born and raised on the Institute farm and was known to have lived all his life on bread and cereals, after operation advantage was taken of the opportunity to test the effects of fasting and fat diets, as will be described in a subsequent paper on acidosis. Neither glycosuria nor acidosis occurred, and the plasma sugar on Oct. 2 was 0.128 per cent, on Oct. 3, 0.092 per cent, and on Oct. 5, 0.101 per cent. After Oct. 5 the dog was kept on rather low protein-fat diets, maintaining a body weight of 10.5 to 10.2 kilos.

Oct. 29. A pan of bread and soup was fed, causing the excretion of 2.39 per cent sugar in 663 cc. of urine.

TABLE III.

Dog D4–28.

Date.	Urine.		Remarks.
	Total nitrogen.	Ammonia nitrogen.	
1917	*gm.*	*gm.*	
Jan. 28	2.14	0.08	
" 29	3.73	0.19	
" 30	1.34	0.11	
" 31	2.33	0.11	
Feb. 1	1.55	0.10	
" 2	2.98	0.30	Total nitrogen in the feces for the period 4.46 gm.
" 3	2.62	0.27	

Oct. 30. A diet was fed of 200 gm. of beef lung, 200 gm. of suet, and 75 gm. of bread, without glycosuria. The plasma sugar before feeding was 0.184 per cent. Taken at 2 hour intervals for 12 hours after feeding it was 0.184, 0.200, 0.222, 0.213, 0.184, and 0.172 per cent. The following morning before feeding it was 0.179 per cent.

Nov. 2. The diet was 200 gm. of lung, 150 gm. of lard, and 100 gm. of bread. The plasma sugar before feeding was 0.143 per cent. Taken at 2 hour intervals for 12 hours thereafter it was 0.166, 0.180, 0.200, 0.208, 0.182, and 0.182 per cent, without glycosuria.

Nov. 3, 10 a.m. Plasma sugar 0.148 per cent. The dog was then fed only 200 gm. of lung. 5 p.m. Plasma sugar 0.143 per cent.

Beginning Nov. 4, on a diet of 200 gm. of lung and 100 gm. of suet, the dog gradually gained weight up to 11.3 kilos on Dec. 4. On that day faint glycosuria appeared, and was absent the next day. On Dec. 6 there was excretion of 0.74 per cent glucose in 124 cc. of urine, on Dec. 7, 0.59 per cent in 136 cc. of urine, and

on Dec. 8, 1.81 per cent in 396 cc. of urine, with a trace of acetone. With 1 day of fasting there were only traces of sugar and acetone, and a 2nd fast day cleared up both. Then the feeding of 100 gm. of suet failed to bring back either sugar or acetone. These remained absent thereafter on a diet of 100 gm. of lung and such

TABLE IV.

Dog D4–28.

Date.	Urine.		Remarks.
	Total nitrogen.	Ammonia nitrogen.	
1917	*gm.*	*gm.*	
Apr. 10	1.69	0.10	
" 11	1.88	0.27	
" 12	1.24	0.10	
" 13	2.40	0.19	
" 14	2.46	0.25	
" 15	1.60	0.16	
" 16	1.28	0.12	
" 17	3.32	0.28	
" 18	1.97	0.20	Total nitrogen in the feces for the period
" 19	2.08	0.22	1.69 gm.

TABLE V.

Dog D4–28.

Date.	Urine. Total nitrogen.	Date.	Urine. Total nitrogen.
1917	*gm.*	*1917*	*gm.*
Oct. 5	1.12	Oct. 13	—
" 6	1.64	" 14	2.18
" 7	2.04	" 15	2.06
" 8	1.78	" 16	1.82
" 9	1.74	" 17	1.60
" 10	2.36	" 18	1.96
" 11	2.18	" 19	3.24
" 12	—	" 20	2.52

small quantities of suet as the dog chose to eat. Dec. 27. Plasma sugar 0.084 per cent, with hemoglobin (Fleischl-Miescher) 114 per cent. Jan. 18, 1917. Plasma sugar 0.083 per cent, with hemoglobin 111 per cent. The weight during this time up to Jan. 27 ranged from 10.4 to 11 kilos.

Jan. 27 to Feb. 3. A metabolism experiment was conducted on a diet of 100 gm. of lung and 100 gm. of suet. The dog was catheterized on these two dates,

but in the interim passed urine spontaneously and regularly. Feces were combined and analyzed in one lot for the entire period. The results are given in Table III.

Beginning Mar. 10, at a body weight of 10.4 kilos, the above diet was increased by 25 gm. of bread. After Mar. 13, this was changed to 50 gm. of lung, 50 gm. of bread, and 100 gm. of suet. Apr. 10 to 19. Urinalyses were performed on the latter diet (Table IV).

The body weight gradually rose from 10 kilos on Mar. 20 to 10.8 kilos on June 30. June 15. The plasma sugar before feeding was 0.081 per cent; 4 hours after feeding, 0.161 per cent. Suet was then omitted; this made the diet only 50 gm. of lung and 50 gm. of bread. The weight was thus reduced by July 17 to 9.75 kilos. After that, 50 gm. of suet were resumed, but were omitted at times as the dog tired of it, so that the body weight was kept between 9.2 and 10 kilos. Oct. 5 to 20. A series of urinalyses, omitting feces, was performed on the usual diet of 50 gm. of lung, 50 gm. of bread, and 50 gm. of suet (Table V).

Nov. 26. The above diet was fed about noon. 3 p.m. Plasma sugar 0.118 per cent. Beginning Nov. 27, bread alone was fed, in increasing quantities, from 150 gm. on the 1st day to 300 gm. on Nov. 30, without glycosuria.

Dec. 1, 12.30 p.m. The dog was fed 300 gm. of bread with 100 gm. of glucose, without glycosuria. The plasma sugar before feeding was 0.13 per cent, and at 4 p.m., 0.216 per cent. Dec. 2. Diet the same. Dec. 3. The glucose was increased to 150 gm., still without glycosuria. Dec. 4. Plain bread and soup mixture was fed. Dec. 5 to 7. 200 gm. of glucose were added daily, with glycosuria never above 0.61 per cent. Dec. 8. The plasma sugar was 0.161 per cent, and the diet was changed to lung *ad libitum*. The body weight at this time was 9 kilos.

Beginning Dec. 14, 100 gm. of suet were added, and on 2 days, *viz.* Dec. 14 and 26, a pan of bread and soup was allowed in addition, without glycosuria. The weight thus rose to 10.8 kilos.

A test with carbohydrate was then performed, in comparison with the one a month previous at a lower weight. Glycosuria was absent on bread diet. On Jan. 3, 1918, when 300 gm. of bread with 100 gm. of glucose were fed to compare with Dec. 1, the plasma sugar before feeding was 0.154 per cent and at 4 p.m., 0.452 per cent, with excretion of 3.8 per cent sugar in 700 cc. of urine for the 24 hours.

Jan. 4. Glycosuria ceased on a diet of 300 gm. of lung and 100 gm. of suet. Jan. 5. On feeding plain bread and soup there was excretion of 3.45 per cent sugar in 415 cc. of urine.

Thereafter the diet of 50 gm. of lung, 50 gm. of suet, and 50 gm. of bread was resumed. A gradual fall in weight ensued, to 9.75 kilos on Jan. 28, 8.75 kilos on Feb. 21, 8.3 kilos on Mar. 11, and 7.4 kilos on June 11. Glycosuria was constantly absent, and up to June 8 the dog maintained the utmost activity. The urine, as collected under the cage, then became increasingly bloody. The cause

was supposed to be bloody diarrhea, due to deficient diet, as described by Rosenheim. The diet was therefore enriched with an abundance of fresh meat, bones, milk, eggs, and yeast, to supply any missing elements. Nevertheless, the condition became worse. June 12. The animal was found dying and was killed for autopsy. The plasma sugar of the heart's blood was 0.159 per cent, the carbon dioxide capacity 32.8 volumes per cent, without acetone or other evidence of acidosis.

Autopsy.—The cause of death was found to be urinary obstruction from a large calculus impacted in the urethra near the bladder, with several smaller stones lodged behind it. More or less urine had been passing up to the day of death, but the bladder and both ureters and kidneys were distended with bloody urine, which had also infiltrated extensively about the neck of the bladder. The intestine was normal throughout and there was no blood in its contents anywhere. The other viscera were negative. The pancreas remnant, normal in appearance and consistency, weighed 3.5 gm.

Microscopic Examination.—The kidneys were inflamed and infiltrated with mononuclear cells chiefly in the cortex and polymorphonuclears chiefly in the medulla. The Armanni or Ehrlich vacuolation was also evident in some tubules. The liver was normal and its cells contained no visible fat. The pancreas remnant was entirely normal and free from fibrosis. The acini were regular and well filled with zymogen, and the islands normal in number and size and free from vacuolation. The other organs were normal.

As the experiment was a long one, the opportunity was taken to use it for several purposes simultaneously. Aside from the acidosis observations mentioned, the following points were included. As low protein diets, sometimes in conjunction with undernutrition, are an occasional feature of diabetic treatment, it was desirable to test the feasibility of such a program in the dog. This was especially desirable in regard to any possible doubts still persisting from the work of Munk,[11] Rosenheim,[12] and Zuntz and Magnus-Levy,[13] though it seems generally accepted that the difficulties reported by these investigators were due not to protein deficiency but to other unsuitable features in the diets used. One of these features is considered to be the use of meat powder in Munk's 10 week experiment. Rosenheim noticed decline of appetite and digestion within 8 weeks, perhaps

[11] Munk, I., *Arch. Physiol.*, 1891, 338; abstracted in *Jahresb. Fortschr. Thier-Chem.*, 1892, xxi, 365.

[12] Rosenheim, T., *Arch. Physiol.*, 1891, 341; abstracted in *Jahresb. Fortschr. Thier-Chem.*, 1892, xxi, 365.

[13] Zuntz, N., and Magnus-Levy, A., *Arch. ges. Physiol.*, 1891, xlix, 438.

due to the rice used, and then obtained a fatal result by changing to an excessive fat ration. The dog described above (No. D4-28) had presumably some tendency to impaired digestion created by the removal of $\frac{1}{1}\frac{1}{2}$ of the pancreas, yet suffered none of the disturbances described by these authors. A little bone-meal daily prevented diarrhea and presumably contributed some mineral salts. From March 14 to November 27, 1917, the dog remained in excellent health and spirits on 50 gm. of beef lung (cooked), 50 gm. of bread, and 50 to 100 gm. of raw beef suet. Weight was gained whenever the calories were sufficient and was lost in normal manner whenever the dog tired of too much suet. After January 6, 1918, there was a gradual and regular loss of weight up to June, on the undernutrition diet of 50 gm. of lung, 50 gm. of bread, and 50 gm. of suet. During these long periods there was no sign of any specific disorder due to the reduction of urinary nitrogen to approximately 2 gm. daily.

During this same time, the animal served as a control for other dogs on high fat diets, as will be described in a later paper.

The influence of body weight upon assimilation was twice observed; namely, the absence of glycosuria on 200 gm. of lung, with fat and sometimes 75 or 100 gm. of bread up to November 4, 1916, at weights below 10.5 kilos, and the occurrence of glycosuria a month later on 200 gm. of lung and 100 gm. of suet at a weight of 11.3 kilos; likewise the lower tolerance of early January, 1918, at a weight of 10.8 kilos, as compared with a month preceding at a weight of 9 kilos. The weight was finally allowed to fall very low, with a view to demonstrating a still higher tolerance, and then ultimately bringing back diabetes by fattening; but this plan could not be carried out because of the accidental death.

This dog was characterized throughout by oliguria and high renal thresholds for sugar. There is a question of the possible existence and injury of renal calculi from the outset, but the appearance of the kidneys did not suggest a very long standing trouble. Similar peculiarities of function have been observed in other dogs under similar conditions, and the conclusions regarding diabetes are supported by blood as well as urine analyses.

The experiment also served as a test of the specific influence of preformed carbohydrate as a cause of injury to the assimilation in dia-

betes. An animal was chosen with potentially severe diabetes, produced by removal of $\frac{1}{1}\frac{1}{2}$ of the pancreas and demonstrated by the occurrence of glycosuria on December 4 to 8, 1916, on a diet of only 200 gm. of lung and 100 gm. of suet, when the body weight had, as stated, been built up to a level which was still subnormal. Under these conditions the dog was undernourished by means of a mixed diet containing an appreciable quantity of carbohydrate, and the experiment was continued long enough to test whether carbohydrate would prove itself a specific poison to the islands of Langerhans. The actual result was a marked gain in tolerance. This is best seen by comparison of the above mentioned glycosuria in December,1916, on 200 gm. of lung and 100 gm. of suet at a weight of 11.3 kilos, with the tests a year later when 300 gm. of bread could be taken without glycosuria at a weight of 10.8 kilos, and when the glycosuria which was produced by the addition of glucose cleared up (January 4, 1918) on 300 gm. of lung and 100 gm. of suet. It may be concluded that the tolerance created by undernutrition was genuine, and that the feeding of carbohydrate within the limits of this tolerance did not damage the assimilation or the islands of Langerhans.

Apart from the above details, the main point of the experiment was to determine whether downward progress is inevitable in an animal with potentially severe diabetes, so that symptoms must ultimately occur in spite of dietary restriction. This dog was kept for approximately $1\frac{3}{4}$ years. Besides the gain in tolerance during this time, the intact state of the islands furnished additional evidence that no injurious change was in progress, and the indications are that the same condition of health could have been maintained indefinitely.

EXPERIMENTAL STUDIES ON DIABETES.

Series I. Production and Control of Diabetes in the Dog.

5. Various Failures of Dietetic Treatment, and Their Causes.

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Plates 65 and 66.

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Glycosuria Controllable Only by Fatal Undernutrition, Because of Severity of Diabetes.

Dog B2–28.—Male; mongrel; black and white; age 1 year; slightly thin; weight 11 kilos. Dec. 18, 1913. Removal of pancreatic tissue weighing 32 gm. Remnant about main duct estimated at 2 gm. ($\frac{1}{17}$). Glycosuria of 0.55 per cent followed the operation, but diminished, and by Dec. 23 was absent. Continued attempts were then made to nourish the animal with low protein-fat diets, often subdivided into several meals during the day, but traces of glycosuria recurred each time, requiring further fasting and undernutrition. Feb. 24, 1914. The dog was at the point of death from undernutrition; weight 6.8 kilos; killed for autopsy.

Autopsy.—The pancreas remnant, not obviously sclerosed, weighed 1.2 gm.

Microscopic Examination.—Portions of the remnant showed interacinar pancreatitis, other portions normal parenchyma. The islands generally were few and small but not to the extent of a positively pathological reduction. Appearances of vacuolation were rare and doubtful, but search through many sections revealed a very few distinct examples.

Dog B2-29.—Male; mongrel; white; age 1½ years; good condition; weight 10.6 kilos. Dec. 22, 1913. Removal of pancreatic tissue weighing 27.7 gm. Remnant about main duct estimated at 2.4 gm. ($\frac{1}{12}$–$\frac{1}{13}$). Only faint glycosuria followed the operation. The subsequent undernutrition was more rigid than in Dog B2-28, in the sense that no more than a trace of glycosuria on a single day was permitted at any time. The same inability to develop a tolerance for any living diet was encountered, and by Apr. 7, 1914 the weight had declined to 5.9 kilos. On that day the dog was killed.

Autopsy.—The pancreas remnant, somewhat nodular and atrophic, weighed 1.65 gm. The liver appeared fatty, presumably from the high proportions of fat in the diet. The autopsy was otherwise negative except for emaciation.

Microscopic Examination.—There was moderate fatty infiltration in the liver; no noteworthy changes in the spleen, kidneys, adrenals, testes, thyroid, parathyroids, and hypophysis. The pancreatic parenchyma mostly showed interacinar fibrosis, in the form of light bands traversing it irregularly and often distorting acini. The fibrosis was presumably the result of damage from an acute inflammation following operation; there were no signs of recent or progressive changes. Islands were scarce and small to a markedly pathological degree. In some considerable areas free from fibrosis there was this same scarcity of islands, and the sections were made up of almost unbroken expanses of acini. No vacuolation was visible.

The pancreas remnants in these animals were small, and there was apparently further destruction of islands by inflammation. Diabetes was more or less completely suppressed by fasting and undernutrition, but as neither dog became able to tolerate a diet sufficient to support life, they both died in a little over 2 months of inanition. Very slight hydropic changes were demonstrable in the islands in the first dog, which had shown frequent traces of glycosuria, but none were found in the second dog, in which glycosuria had been more rigorously controlled. These examples are comparable with a few human cases of great severity, especially when the treatment consists in continual attempts to push the diet to the limit of tolerance.

Originally Mild Diabetes; Death from the Undernutrition Made Necessary by Prolonged Slight Overfeeding.

Dog B2-71.—Female; mongrel; yellow; age 3 years; good condition; weight 14.7 kilos. June 3, 1914. Removal of pancreatic tissue weighing 19.2 gm. Remnant about main duct estimated at 2.25 gm. ($\frac{1}{9}$). No glycosuria ensued on meat diet. On change to bread and soup, there was the usual single day of glycosuria, thereafter none. The following tolerance tests were performed while the animal was at a fairly constant weight of 13 to 13.5 kilos, no food being given on the test days.

June 26. Subcutaneous injection of 42 gm. of Merck anhydrous glucose in 30 per cent solution (3 gm. per kilo on basis of normal weight of 14 kilos). No glycosuria. July 8. Subcutaneous injection of 56 gm. of Merck glucose in 30 per cent solution (4 gm. per kilo on basis of 14 kilo weight). No glycosuria. July 17. Subcutaneous injection of 84 gm. of Merck glucose in 30 per cent solution (6 gm. per kilo on basis of 14 kilo weight). Glycosuria 0.3 per cent in 10 cc.

of urine; thereafter negative. July 30. 56 gm. of Merck glucose in 30 per cent solution given by stomach tube (4 gm. per kilo on basis of 14 kilo weight). No glycosuria. Aug. 12. 84 gm. of Merck glucose in 30 per cent solution given by stomach tube (6 gm. per kilo on basis of 14 kilo weight). 3 hours after dose, moderate sugar reaction in 1 cc. of urine from bladder; next morning trace in 420 cc.

The subsequent record is contained in Table I.

TABLE I.

Dog B2-71.

Date.	Body weight.	Diet.	Remarks.
1914	*kg.*		
Aug. 21–23	13.8	Bread and soup with 100 gm. of glucose daily.	No glycosuria.
" 24–26	13.9	Bread and soup with 200 gm. of glucose daily.	" "
" 27–Sept. 26	14–11	Bread and soup with 300 gm. of glucose daily.	Glycosuria varying from heavy to negative; generally slight. Diarrhea; decline of weight and strength.
Sept. 27–Oct. 27	11–12	Bread and soup *ad libitum*.	Occasional traces of glycosuria, governed by variations of appetite, increasing with the slight gain in weight.
Oct. 28–Nov. 7	12.1–11.8	Bread and soup first with 50, then with 100 gm. of glucose daily.	Heavy glycosuria, diminishing as appetite failed.
Nov. 8–Jan. 2, 1915	11.8–13.8	Bread and soup with a little meat and suet daily.	Glycosuria absent at first, then irregular traces, becoming heavy at end with gain in weight.
1915			
Jan. 3–6	13.8–13.1	Fasting.	Glycosuria absent.
" 7–Mar. 28	13.5–12.6	Beef lung *ad libitum*.	" "
Mar. 29–July 2	12.6–10.8	1 kilo of beef lung daily.	No glycosuria except on days of carbohydrate feeding, as recorded in Paper 2,* which showed tolerance between 100 and 200 gm. of bread or rice.
July 3–Aug. 3	10.8–12.5	Beef lung *ad libitum* with 50 gm. of suet daily.	No glycosuria except in occasional experiments, which showed that tolerance was less than 50 gm. of bread.

* Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 399.

The dog was then used for experiments unrelated to the present subject. Prior to death on Nov. 24, 1915, glycosuria was beginning to appear on a carbohydrate-free diet of beef lung and suet.

Autopsy.—The important feature of the autopsy was the pancreas remnant, which weighed 4.1 gm.

Microscopic Examination.—The islands were unusually large and numerous, and contained a slight sprinkling of maximally vacuolated cells, evidently related to the overfeeding preceding death.

The record represents downward progress during approximately a year and a half of life after operation. The animal was never subjected to any heavy continuous glycosuria. Certain fluctuations of tolerance were related to changes of weight, as noted in Table I, but there was no obesity and the weight remained always below the original normal level. The dog was merely kept fed as close as possible to the limit of tolerance, up to January, 1915, on bread, thereafter on meat, and tests or experiments were frequently interpolated producing slight or transitory glycosuria. Slow decline of assimilation thus occurred notwithstanding hypertrophy of the pancreas remnant and its richness in islands.

This finding, together with a number of animal and human observations, suggests a functional injury or defect in the islands when their number and size seem sufficient to prevent diabetes. The degree of vacuolation as usual corresponded to the glycosuria.

The chief point in the experiment is its close imitation of human cases in which the diet is forced to the verge of tolerance and the patient commits occasional indiscretions causing transitory glycosuria. The familiar downward progress of such cases is accurately reproduced.

Dog B2-31 (*Fig. 4*).—Female; bull-terrier; white; age 2 years; good condition; weight 11 kilos. Dec. 23, 1913. Removal of pancreatic tissue weighing 19 gm. Remnant about main duct estimated at 2.6 gm. ($\frac{1}{3} - \frac{1}{5}$). The dog was able to live on bread and soup with only slight intermittent glycosuria. Her gluttony and fondness for sugar made diabetes possible, as described in Paper 2.[1]

Mar. 8, 1914. Fasting was begun. Mar. 12. Glycosuria was absent. Nevertheless, the fast was continued through Mar. 16. Mar. 17. The body weight was 8 kilos, and very low weighed diets were begun, generally 50 gm. of lung and

[1] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 382.

50 gm. of suet, reducing the weight to 7.4 kilos on Mar. 25. These diets also were given in the form of meals spaced as far apart as possible, in the hope of better utilization from the standpoint of diabetes and also of the protein economy in the sense of Thomas,[2] though no plain evidence of the benefit of such a course was ever seen. Beginning Apr. 2, the daily ration was increased to three meals of 30 gm. of lung and 30 gm. of suet each. By Apr. 24 the weight had risen to 9.3 kilos, and the diet was increased to 40 gm. of lung and 40 gm. of suet thrice daily.

Apr. 27, 4 p.m. A tolerance test was performed by subcutaneous injection of 11 gm. of Merck anhydrous dextrose (1 gm. per kilo on the basis of the normal weight of 11 kilos) in 30 per cent solution. 10 p.m. Urine by catheter was 128 cc. with 0.9 per cent glucose. Apr. 28, a.m. Urine by catheter was 388 cc. with 0.4 per cent glucose; *i.e.*, an excretion of 2.7 gm. and utilization of 8.3 gm. of the dose.

Glycosuria was then absent till May 1, when the body weight was 9.9 kilos. It was continuous for 3 days, May 1 to 3, but not above 0.4 per cent. After fast days on May 3 and 4, the same diet was resumed, with glycosuria absent except as follows: May 7, 0.2 per cent, stopped by a fast day; May 17, 0.3 per cent, stopped likewise; May 24, 0.5 per cent, stopped by 2 fast days. After this the diet was reduced to 20 gm. of lung and 30 gm. of suet thrice daily. Nevertheless, on May 30, at a body weight of 9.75 kilos, there was a return of 0.17 per cent glycosuria. Though this ceased within 24 hours, the fast was continued for 5 days.

Thereafter, on a diet of 30 gm. of lung and 30 gm. of suet thrice daily, there was a gradual gain of weight up to 10.8 kilos on July 3, when there appeared glycosuria of 0.46 per cent in 600 cc. of urine, increasing to 0.97 per cent in 835 cc. of urine on July 4.

Fasting was imposed till July 7, when the weight was 10.3 kilos. Thereafter 100 gm. of lung were fed daily. Though the weight fell slightly on this lower caloric diet, there was continuous slight glycosuria on July 11 to 13, not above 0.66 per cent. The diet was then changed to 100 gm. of fresh beef pancreas daily, and the same glycosuria (not above 0.7 per cent) continued till July 20, again illustrating the uselessness of pancreas feeding.

After a 4 day fast, the weight was down to 9.3 kilos on July 24, and the diet of 100 gm. of raw pancreas was resumed, together with 30 gm. of bone-meal, which was commonly used for such experiments, as previously stated. In addition to the usual purpose of preventing diarrhea and supplying salts to the body, the bone-meal might perhaps serve in this connection to neutralize gastric juice. July 28. There began an excretion of 1.5 per cent glucose in 425 cc. of urine, increasing to 4 per cent in 360 cc. of urine the next day. Fasting then stopped the glycosuria in 2 days, but was continued to Aug. 4, when the weight was 8.5 kilos. The diet of 100 gm. of pancreas was then resumed, with resultant glyco-

[2] Thomas, K., *Arch. Physiol.*, 1910, suppl., 249.

suria of 0.2 to 0.4 per cent on Aug. 6 to 8. Fasting was then instituted for 4 days. As it seemed evident that the rigorous undernutrition was beginning to be effective in diminishing glycosuria, a change was made to lung diet, to guard against ascribing any benefit incorrectly to the pancreas feeding. Accordingly, beginning Aug. 11, 100 gm. of lung were fed daily, with absence of glycosuria except for a trace on 1 day. The weight at this time was 8.15 kilos.

Aug. 18. Bread and soup were given by mistake, with resultant glycosuria of 6.6 per cent in 1,275 cc. of urine. Fasting till Aug. 25 was necessary before glycosuria ceased. Thereafter on a diet of 20 gm. of lung and 20 gm. of suet thrice daily, glycosuria was absent except for a trace on 1 day.

Sept. 11. As the dog had tired of fat, the diet was changed to 50 gm. of lung thrice daily. Sept. 13. Glycosuria of 1 per cent appeared, the higher protein as usual having a greater immediate effect than the higher calories. Sugar excretion was continuous on this diet, and by Sept. 26 had risen to 6 per cent. Accordingly, fasting was begun on Sept. 27; glycosuria was absent on Oct. 4; nothing more than 60 gm. of suet was given daily till Oct. 9, when a diet of 50 gm. of lung and 50 gm. of suet was begun, and increased on Oct. 16 to 80 gm. of lung and 80 gm. of suet. The weight was then 6.7 kilos.

Slight glycosuria, not above 0.5 per cent, occurred on Oct. 22, 23, 28, 29, 30, and Nov. 6. Suet was then omitted, but similar sporadic traces continued till Nov. 23, when 2 fast days were imposed, followed by a diet of 50 gm. of lung and 30 gm. of suet. The weight rose slightly, to 6.9 kilos on Dec. 9, on which day glycosuria of 0.6 per cent appeared.

With 2 days of fasting, the urine was sugar-free on Dec. 11. Dec. 12. A diet of 50 gm. of lung was begun, with the addition of 30 gm. of suet after Dec. 18. About this time the previously lively animal began to fail seriously. The digestion began to be impaired, as usual in cachexia, and by Dec. 31 the weight was down to 5.8 kilos. The diet was changed to 50 gm. of raw pancreas and 30 gm. of suet, in the hope of improving digestion, but the pancreas was of no avail even for this purpose. Liberal feeding with pancreas and fat was attempted on Jan. 2 and 3, 1915; the result was a glycosuria of 1 per cent on Jan. 4, while the weight had fallen to 5.3 kilos. The dog was chloroformed.

Autopsy.—The urine in the bladder was 13 cc., containing 0.53 per cent glucose, a trace of albumin, and no acetone. Except for emaciation and corresponding atrophy of viscera, the autopsy was negative as usual. The weights of the principal organs were as follows: liver 101 gm.; both kidneys 48.8 gm.; both adrenals 2 gm.; pancreas remnant 3.8 gm. The pancreas remnant was soft, lobulated, and in places semitranslucent.

Microscopic Examination.—The liver showed a considerable sprinkling of glycogen granules by Best's carmine stain; these were located in the periphery of the lobules, the centers containing none; fat was scanty. The acinar tissue of the pancreas was normal. Islands were remarkable for both fewness and smallness. Their cells were normal in appearance and no vacuolation was discoverable.

Notwithstanding the extreme emaciation and prostration, glycosuria evidently continued till death. The point is mentioned because dogs of this type herein correspond to the rule for human cases. In some totally depancreatized dogs authors have reported cessation of glycosuria before death.

In spite of the severity of diabetes thus indicated, no sign of hydropic degeneration was visible in the islands of Langerhans. Study of a sufficient series of animals gives a simple explanation. The overstimulation causing such visible degeneration is intense and the cellular disintegration rather rapid. The diabetes in this dog had been largely controlled by diet. Life had continued for more than a year, and an active hydropic process must have destroyed all the islands before this time. The milder degree of overstimulation and longer clinical course should naturally give rise to a slower island destruction, in the form of occasional loss of cells, which would seldom be demonstrable microscopically. This supposition, which in animals can be fully verified by observations of all types, degrees, and stages of the process, serves to explain the similar findings in human cases.

The principal reason for the detailed record given is to show the exact similarity to the clinical course of many human cases. The animal started with mild diabetes; in fact, the large size of the pancreas remnant and the subsequent hypertrophy would probably have permitted a cure had opportunity been given. Gluttony and carbohydrate excess made the diabetes severe. A familiar plan of treatment was then employed, in the form of a protein-fat diet pushed to the point of maintaining the highest possible weight and strength. There was the usual onset of glycosuria with gain in weight, the necessity for repeated fasting periods, and the gradual decline of tolerance, weight, and strength. This occurred without any breaking of diet on the part of the subject. The two principal features of cases under such management are thus illustrated; first, life and strength are preserved much longer than when active diabetic symptoms are allowed to continue; second, the alleged "spontaneous" downward progress of human patients occurs in typical slow but sure form.

Glycosuria Uncontrollable after Protein Overfeeding.

Dog B2–56.—The record of this animal was given in Paper 3[3] as an example of downward progress on excessive protein diet. The attempt to control the condition by fasting beginning May 14, 1914, gave results shown in Table II.

The cachexia was unusually rapid in progress, and even before the fast the dog had lost considerable hair and was developing ulcers at points of bony pressure, especially over the ischiatic tuberosities and

TABLE II.

Dog B2–56.

Date.	Urine.		Remarks.
	Volume.	Glucose.	
1914	*cc.*	*per cent*	
May 14	840	1.7	Weight 13.5 kilos. Fasting.
" 15	490	3.0	Fasting.
" 16	530	3.5	"
" 17	415	1.6	"
" 18	450	1.0	"
" 19	360	1.5	"
" 20	267	1.4	"
" 21	397	1.5	"
" 23	430	1.3	"
" 24	535	2.4	Weight 9.9 kilos. Diet of meat *ad libitum* begun because of dangerous weakness.
" 25	1,000	0.7	Diet of meat *ad libitum*.
" 26	1,150	0.8	" " " " "
" 27	690	0.6	" " " " "
" 28			Weight 9.7 kilos. Moribund. Killed for autopsy.

the joints of the limbs. These were foul and spreading in character, without tendency to heal. The one at the right elbow perforated into the joint and was probably the origin of the large axillary abscess of creamy pus found at autopsy. It is unknown why a few partially depancreatized dogs show cachexia and susceptibility to infection almost like totally depancreatized animals. The "gangrene" and lowered resistance of human patients are fully reproduced in such animals. Also in occasional human cases the diabetes is evidently too severe to be controlled by fasting.

[3] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 560.

Glycosuria from Prolonged Protein-Fat Overfeeding, Checked at First by Short Fasting, Later Uncontrollable.

Dog B2–57.—Female; mongrel; yellow; age 5 years; good condition. May 4, 1914. Received at normal weight of 11 kilos. After reduction of weight to 8.65 kilos by fasting, on May 21 partial pancreatectomy was performed; 19.6 gm. of tissue removed, and remnant left estimated at 3 gm. The dog was used to test the effects of fat feeding and obesity. The first continuous glycosuria began Dec. 24, at a weight of 16.3 kilos. This persisted till stopped by fasting, Jan. 4 to 6. The record for 1915 is given in Table III.

Dog B2–30.—Female; mongrel; yellow; age 1½ years; good condition; weight 10 kilos. Dec. 22, 1913. Removal of pancreatic tissue weighing 19.5 gm. Remnant about main duct estimated at 2.5 gm. Glycosuria was absent after operation till milk was fed on Dec. 26. It then remained heavy (up to 3.3 per cent) on bread and soup diet till Jan. 1, 1914, after which a large (unweighed) admixture of glucose was required to produce glycosuria. The dog gradually reached the point of refusing the mixture absolutely; therefore on Jan. 17 the diet was changed to chopped meat mixed with lactose, which was eaten abundantly and maintained glycosuria as high as 5 per cent. After Jan. 22 heavy glycosuria continued on a diet of meat only. Feb. 2. Fasting was begun in the attempt to stop glycosuria (Table IV).

Blood Transfusion.—Feb. 20. Transfusion was undertaken partly in an attempt to increase strength and partly to determine whether, when everything possible was done by fasting, there might be any transfer of pancreatic hormone in the blood sufficient to check the diabetes. 4.30 p.m. Urine under the cage was 25 cc., containing glucose 4.8 per cent. When all was ready for transfusion, catheterization yielded 2 cc. of urine, with glucose 6.2 per cent. A large needle was then inserted into an external jugular vein, and by the use of vaseline-coated syringes without anticoagulants blood was drawn from a normal dog and injected into this dog to the amount of 350 cc. by 5.30 p.m. At this time the dog showed collapse, passed urine and feces, and seemed on the point of death, from toxic action of the infused blood or simple overfilling of the circulation. The transfusion was therefore ended, and the emptiness of the bladder verified with the catheter. The total urine for the transfusion period was 3 cc., containing 6.4 per cent glucose. 6.15 p.m. The urine obtained by catheter was 4 cc., containing 3 per cent glucose and slight albumin. The dog drank thirstily. 8.30 p.m. The urine by catheter was 19 cc., with a faint trace of albumin and 1.7 per cent sugar. 2.30 a.m. The urine by catheter was 26 cc. with 2.5 per cent glucose and the same faint albumin. The mere traces of glycosuria on the succeeding days, as shown in Table IV, may appear striking, but the writer is inclined to interpret them as accidents of renal permeability rather than benefit to the diabetes.

TABLE III.

Dog B2–57.

Date.	Body weight.	Diet.	Urine.	
			Volume.	Glucose.
1915	*kg.*		*cc.*	*per cent*
Jan. 1	16.3	100 gm. of lard and 1,000 gm. of beef lung, the latter not all eaten.	650	1.5
" 2		100 gm. of lard and 1,000 gm. of beef lung, the latter not all eaten.	880	4.2
" 3		Most of food uneaten.	890	1.6
" 4		Fasting.	180	0.7
" 5	15.4	"	339	0.22
" 6		"	261	0
" 7	14.8	"	113	0
" 8	14.5	"	56	0
" 9	14.3	100 gm. of lung.	196	0
" 10		200 " " "	194	0
" 11	14.0	300 " " "	231	Doubtful.
" 12	14.13	400 " " "	280	0
" 13	13.73	500 " " "	203	0
" 14	13.55	500 " " "	186	0.18
" 15	13.60	600 " " "	239	1.37
" 16	13.75	700 " " "	107	1.33
" 17		Fasting.	81	0
" 18	13.30	"	65	0
" 19	13.20	500 gm. of lung.	119	0.7
" 20	13.05	500 " " "	369	0.23
" 21	12.86	Fasting.	165	Doubtful.
" 22	12.60	"	154	0
" 23	12.45	400 gm. of lung.	250	0.20
" 24		400 " " " 30 gm. of suet.	386	2.50
" 25	12.40	Fasting.	14	0.90
" 26	12.18	"	99	0.60
" 27	12.06	"	95	Very faint.
" 28	11.90	"	50	0
" 29	11.75	100 gm. of lung.	170	0
" 30	11.75	150 " " "	350	2.80
" 31		Fasting.	273	2.00
Feb. 1	11.30	"	142	1.50
" 2	11.15	"	211	0.80
" 3	11.10	"	195	1.00
" 4	10.85	"	185	0.70
" 5	10.68	"	81	0.60
" 6	10.61	"	89	2.00
" 7		"	183	1.25

TABLE III—*Concluded.*

Date.	Body weight.	Diet.	Urine.	
			Volume.	Glucose.
1915	*kg.*		*cc.*	*per cent*
Feb. 8	10.25	Fasting.	176	3.00
" 9	10.10	"	204	2.78
" 10	9.90	"	185	2.00
" 11	9.80	"	70	2.00
" 12	9.50	"	116	1.20
" 13	9.50	50 gm. of glidine.	295	3.03
" 14		Fasting.	135	1.40
" 15	9.30	"	102	1.70
" 16	9.15	"	105	2.50
" 17	9.00	"	175	2.50
" 18	8.77	40 gm. of extract of beef.	385	2.53
" 19	8.78	Fasting.	274	1.33
" 20	8.85	50 gm. of lard; 50 gm. of meat.	260	1.92
" 21		50 " " " 50 " " "	120	1.41
" 22		50 " " " 50 " " "	219	0.72
" 23	8.25	50 " " " 50 " " "	570	0.30
" 24	8.10	50 " " " 50 " " "	800	0.69
" 25	8.10	50 " " " 50 " " "	752	0.72
" 26	7.85	50 " " " 50 " " "	747	0.80
" 27	7.80	50 " " " 100 " " "	1,295	0.90

Subsequent History of Dog B2–30.—After Mar. 21, the dog was given 25 gm. of lung and 25 gm. of suet two or three times daily; *i.e.*, a diet of either 50 or 75 gm. each of lung and suet. Apr. 2. At a body weight of 5.5 kilos, glycosuria of 2.7 per cent appeared, and was stopped only by fasting till Apr. 5. The diets thereafter were lower than before, sometimes only 10 gm. of lung and 10 gm. of suet thrice daily. With advancing cachexia the digestion became poor; glycosuria was absent, but by Apr. 21 the weight had fallen to 4.3 kilos, and the animal could barely stand. Accordingly, on that date it seemed necessary to give four meals of 40 gm. of lung each, without suet. The result was an immediate glycosuria of 1.7 per cent in 140 cc. of urine. Though the diet was increased to 50 gm. of lung four times daily, which was as much as the dog would take, glycosuria ceased through failure of digestion. Apr. 24. The animal was at the point of death and was chloroformed.

Autopsy.—Performed immediately. Aside from the extreme emaciation and accompanying atrophy of viscera, the autopsy was negative. The pancreas remnant, normal in appearance and consistency, weighed 3.9 gm.

Microscopic Examination.—The acini were normal, uneven in the degree of filling, but mostly containing abundant zymogen. Island tissue was scarce

TABLE IV.

Dog B2–30.

Date.	Body weight.	Urine.		Dextrose-nitrogen ratio.
		Glucose.	Nitrogen.	
1914	*kg.*	*gm.*	*gm.*	
Feb. 2	8.6	9.25		
" 3				
" 4	8.1			
" 5	7.8	1.25		
" 6	7.8	2.21		
" 7	7.8	3.42	3.14	1.09
" 8		0.92	3.11	0.29
" 9	7.5	1.48	2.81	0.53
" 10	7.25	0.60	2.69	0.22
" 11	7.15	4.45	3.25	1.37
" 12		2.22	2.67	0.83
" 13	6.90	0.06	2.60	
" 14	6.80	Faint.	1.93	
" 15		"	2.48	
" 16	6.55	0.64	3.40	
" 17	6.50	1.02	2.08	0.49
" 18	6.30	0.62	1.80	0.34
" 19	6.20	2.83	1.73	1.63
" 20	6.08	3.68	2.44	1.51
" 21	6.40	Faint.	2.43	
" 22	6.23	"	2.63	
" 23	6.02	4.13	3.82	1.08
" 24		7.32	4.99	1.47
" 25	5.65	2.82	3.53	0.80
" 26	5.60	Faint.	3.41	
" 27	5.43	0	4.66	
" 28	5.45	0	3.30	
Mar. 1	5.35	0	4.44	
" 2	5.20	0	3.01	
" 3	5.18	0	3.07	
" 4	5.20	0	2.42	
" 5	5.38	0	2.74	
" 6	5.28	0.90	3.14	
" 7	5.33	0.24	2.20	
" 8	5.28	Very faint.	2.28	
" 9	5.20	0	1.31	
" 10	5.15	0	1.98	
" 11	5.23	0	2.06	
" 12	5.23	0	1.54	
" 13	5.20	0	1.87	
" 14	5.20	0	1.97	
" 15	5.28	0	1.89	
" 16	5.30	0	1.97	
" 17	5.25	0	1.89	
" 18	5.28	0	2.11	
" 19	5.20	0	2.75	
" 20	5.20	0	2.20	

almost to the vanishing point. Search revealed occasional tiny clumps of cells of island character. No Bensley stains were made, but by comparison with other animals in which such stains were made these surviving, non-vacuolated cells may be interpreted as alpha cells, in confirmation of the original observations by Homans.[4] In rare instances such cell clumps contained one to three maximally vacuolated cells, presumably the last remains of degenerated beta cells. As usual, the islands were not replaced by fibrosis; the tissue merely gave the impression of unbroken expanses of acini.

Starting with sugar-free urine on January 1 on bread and soup diet, this dog showed a downward progress which was so rapid that within 1 month the pancreatic function had fallen too low to support life.

The dextrose-nitrogen ratio was never maximal and was generally low. It is known from the literature that even totally depancreatized dogs do not necessarily show the full 2.8 ratio during fasting. Partially depancreatized dogs of the present type seldom or never show this full ratio during fasting, yet the absence of it is no proof that life can be maintained.

The occasional temporary cessation of glycosuria in this animal during fasting is not unusual under these circumstances and is presumably due to changes of renal permeability. It is probable that the blood sugar remained high till about the time of death.

Transfusion of blood improved the strength slightly and perhaps altered renal permeability temporarily, but had no positive effect upon the diabetes. In view of the similar result in a child,[5] it may be concluded that the effect of transfusion is negative.

The evidence of the hopelessly low pancreatic function was that with the lowest possible diets and body weights, lasting freedom from glycosuria could not be achieved, so that the animal necessarily died from weakness.

The extreme degree of exhaustion and disappearance of island tissue correspond to the lowered function as mentioned.

[4] Homans, J., *J. Med. Research*, 1914, xxx, 49; 1915, xxxiii, 1.

[5] Allen, F. M., Stillman, E., and Fitz, R., Total dietary regulation in the treatment of diabetes, Monograph of The Rockefeller Institute for Medical Research, No. 11, New York, 1919, Chapter III, Case 45, 361.

Dog B2–01 (*Figs. 2 and 3*).—Part of the history of this animal was given in Paper 3. The terminal period so closely resembles that of Dog B2–30 that it can be summarized here very briefly, particularly because it supplements the

TABLE V.

Dog B2–01.

Date.	Urine.						Plasma sugar.	Remarks.
	Volume.	Glucose.		Total nitrogen.	Dextrose-nitrogen ratio.	Nitroprusside reaction.		
1919	*cc.*	*per cent*	*gm.*	*gm.*			*per cent*	
Oct. 22	145	2.20	3.19	4.66	0.68	Heavy.	0.432	Weight 13.3 kilos. Fasting.
" 23		Heavy.				"		Fasting.
" 24	155	1.80	2.79			"		"
" 25		Heavy.				"		"
" 26	65	2.29	1.48			"		"
" 27		Heavy.				"		"
" 28	118	1.15	1.36			"		"
" 29	50	0.95	0.48	0.98	0.49	Moderate.		"
" 30	110	0.45	0.50			"		"
" 31	200	Moderate.				"		"
Nov. 1	200	0.18	0.36	1.72	0.21	Slight.		"
" 2		Slight.				"		"
" 3		Faint.				"		"
" 4		0				"		"
" 5		0				Negative.	0.322	Fed 50 gm. of tallow.
" 6		0				"		" 100 " " "
" 7		0				"		Refuses fat. Fed bones.
" 8	1,500	0.80	12.0	5.6	2.1	Negative.		Fed 100 gm. of beef.
" 9	270	0.55	1.5	3.2	0.47	"		Fasting.
" 10	190	0.25	0.48			Slight.		"
" 11	275	Slight.		1.40		Moderate.		"
" 12	200	"		1.30		Slight.		"
" 13		"				Negative.		"
" 14	210	0.40	0.84	2.03	0.41	"		"
" 15		Slight.				"		"

other by its blood sugar analyses, which were lacking in Dog B2–30. Heavy sugar and acetone reactions being found present on the diet of lung and suet as stated,[6] and the blood plasma on Oct. 16, 1919 showing 0.520 per cent sugar and a slight nitroprusside reaction, fasting was begun on this date in the

[6] Allen,[3] p. 570.

attempt to control the diabetes, though with little expectation of success. After Oct. 22, the seriousness of the condition being confirmed by the stubborn persistence of glycosuria, occasional quantitative analyses of sugar and nitrogen were performed (Table V). Urine was voided without catheterization, and the analyses apply to such daily specimens, not to accurate 24 hour specimens.

Nov. 15, p. m. The dog was dying. An intraperitoneal saline injection given after taking blood at 8 p.m. failed to benefit. 10 p.m. Animal found dead. The results of blood analyses are shown in Table VI.

This animal was one of those in which the severity of diabetes is too great to be checked by fasting. This condition is more common in experimental than in clinical diabetes, but is by no means unknown in the latter. The greater susceptibility of human patients to acidosis

TABLE VI.

Dog B2-01.

Date.	Plasma sugar.	Blood urea per 100 cc.	Plasma chlorides per 100 cc.	Plasma CO_2 capacity.	Plasma nitroprusside reaction.
1919	*per cent*	*mg.*	*mg.*	*vol. per cent*	
Oct. 16	0.520	23.4	550	59.8	Moderate.
" 22	0.432				Slight.
Nov. 5	0.322	26.0	533	56.6	Negative.
" 14	0.624	71.4	562	62.4	Slight.
" 15, 10 a.m.	0.872				Negative.
8 p.m.	0.600	38.6	536	59.4	"

is the probable reason why, with hopelessly severe diabetes, they generally die quickly in coma rather than in the prolonged cachexia which is characteristic of dogs. It will be shown later that under suitable conditions dogs also reach a state where they develop coma with either feeding or fasting.

It is not known whether the D:N ratio in this animal was ever maximal. The high ratio of 2.1 on November 8, in consequence of only a single feeding of 100 gm. of beef, suggests that the full 2.8 ratio might have been present on feeding; but on the other hand, the unusual glycosuria on this date may have represented partly a mere sweeping out of retained sugar. The most striking feature is the remarkably low sugar excretion and D:N ratios in a hopelessly severe case of diabetes. Such submaximal ratios have been the rule in other dogs of this sort during fasting.

Renal impermeability, which is a familiar phenomenon in many human patients, seemed to be largely responsible for the low and variable glycosuria. Absence of glycosuria, on November 4 to 7, with plasma sugar as high as 0.322 per cent, was similar to what has been observed in several other animals under similar conditions, and could not be interpreted as indicating that the diabetes was under control. It may be noted that this impermeability existed in an animal without histological signs of nephritis at autopsy, and with no known cause of impaired renal function other than the diabetes.

The apparent renal impermeability did not include retention of chlorides so far as indicated by the blood analyses. The blood urea rose to a strikingly high figure on November 14, but fell before death in a manner difficult to explain by simple retention. The observations concerning urea also closely reproduce the conditions found in certain human patients.

Nitroprusside reactions were heavy in the urine and slight in the plasma on beginning fasting, and diminished during the fast, as usual in human patients. This clearing of the acetone bodies was approximately parallel with the decline of glycosuria. These tests became negative during the period of freedom from glycosuria, and returned after glycosuria had been restored by protein feeding. They were negative preceding death, as often happens in cachexia. The principal point of these observations is that in this hopelessly diabetic animal the sugar, whether retained from renal impermeability or any other cause, seemed somehow to be used in a way to prevent acetoacetic acid formation.

Dog C3–56 (*Fig. 9*).—Female; mongrel; black and white; age 3 years; moderately well nourished; weight 15.3 kilos. Jan. 27, 1916. Received. Raised a litter of pups in laboratory. July 13. Removal of pancreatic tissue weighing 35 gm. Remnant about main duct estimated at 4.6 gm. (about $\frac{1}{9}$). Glycosuria occurred on bread and soup feeding, but later required glucose for its continuance. The tolerance was thus broken down so that by the middle of Aug. glycosuria was present on a diet of 1 kilo of beef lung. High fat diets were then given, for purposes and with results explained in detail in a previous publication.[7] The table there shown carried the record up to Nov. 4. The condition then was one of persistent and stubborn hyperglycemia without glycosuria,

[7] Allen, F. M., *Am. J. Med. Sc.*, 1917, cliii, 349, 364.

such as excessive fat diets often produce in human patients. The utmost effort was then made to save the animal by diets such as those used for human cases. The diet of 200 gm. of lung daily was continued to Nov. 18, with the purpose of sparing body protein and conserving strength as well as possible, while building up tolerance by undernutrition. On Nov. 18 the plasma sugar was still 0.208 per cent, though the weight had fallen to 7.6 kilos, and the animal was weak. It was therefore deemed necessary to reduce the protein, and the diet was changed to 75 gm. of lung and 75 gm. of suet, with the addition of one raw egg daily to contribute variety and supply any possible need for fat-soluble vitamine. As the appetite failed, other forms of cooked and raw meat were substituted for variety in the same quantity. By Dec. 3 all food was refused. The urine remained free from sugar and acetone as it had been throughout this entire period, but the plasma sugar of the moribund animal was still 0.137 per cent and the carbon dioxide capacity 37.6 volumes per cent, with analyses for all three acetone bodies negative and all other signs of acidosis absent. The dog then weighed 7.25 kilos in a condition of extreme cachexia, with spreading and perforating ulcers on the legs suggestive of diabetic "gangrene." The animal was therefore killed for autopsy.

Autopsy.—The emaciated cadaver contained no visible fat. The liver was small, not fatty, weighing 218 gm. The kidneys, pale and edematous appearing, weighed together 56.5 gm. The viscera were otherwise negative except for atrophy. The pancreas remnant, normal in appearance and consistency, weighed 4.25 gm.

Microscopic Examination.—The organs were negative except for the usual vacuolation of renal tubules due to glycogen or fat. The pancreas remnant, free from fibrosis, consisted of almost unbroken expanses of small acini, some entirely empty but the majority containing zymogen. Only the last remains of exhausted islands were found, in the form of small clumps of cells, sometimes maximally vacuolated (presumably beta cells), sometimes non-vacuolated (presumably alpha cells), sometimes a mixture of the two. There was also vacuolation in the cells of some of the small ducts, as sometimes found in this extreme terminal stage of diabetes.

The experiment illustrates hyperglycemia and impairment of assimilation brought on chiefly by fat feeding, and downward progress notwithstanding absence of glycosuria. Conditions in many human cases are thus closely imitated.

The hydropic degeneration of islands with hyperglycemia without glycosuria is also illustrated. Under these as under all other circumstances, the tendency is for canine diabetes to run a more rapid course than human diabetes. For this reason hydropic degeneration is always easier to demonstrate in dogs, but may be assumed to occur

more slowly in human patients who show a similar but slower downward progress with hyperglycemia.

The clinical and anatomic findings combine to support the view that the internal pancreatic function was here too deficient to support life. A similar conclusion may be drawn from similar evidence in some of the worst human cases.

One point worth mentioning is that diabetic dogs are not necessarily wretched and cachectic. Especially those possessing a maximum of pancreatic tissue, and best suited for therapeutic and some other tests, may be normal appearing and also comfortable and happy. The other point of importance is the degree to which the health and life of these animals is dependent on their diet. These facts are shown more plainly by photographs (Figs. 1 to 12) than by any verbal description, and also the parallelism with the conditions of human patients is more clearly illustrated.

CONCLUSIONS.

1. Practically every detail of clinical diabetes can be reproduced in partially depancreatized animals. The resemblance is made still more exact by the susceptibility of such dogs to acidosis and coma, as will be shown in later papers, and also by the similarity of the anatomic changes in the islands of Langerhans. These animals are therefore useful test objects for a therapeutic investigation.

2. These animals at first show considerable tendency to regain assimilation, comparable to that in the early stages of most human cases of diabetes; and in some instances they recover so as to be able to endure any degree or duration of carbohydrate feeding and can be made diabetic only by removal of additional pancreatic tissue. Similar recovery in some human cases, especially after acute pancreatitis, is a probability. This recuperative tendency can be negatived by overfeeding, even without glycosuria. With duration of the diabetes the power of recuperation diminishes and practically disappears in dogs as in human patients.

3. In the absence of progressive pancreatitis or other extraneous causes, these dogs show no inherent downward tendency in their assimilation. This conclusion rests upon observations as long as 6

years from the first pancreas operation and 3 years of known diabetes. This absence of inherent progressiveness is what should naturally be expected in animals with simple resection of part of an organ, and serves further to fit them for accurate feeding experiments.

4. Every detail of the downward progress of human patients on various diets is reproduced in such animals. They lose assimilation and die most rapidly on diets rich in carbohydrate, and less rapidly on excess of other foods. The differences between sugar and starch, and between starch and protein, seem to be only those of degree and time rather than anything absolute. The important point is that, granting the absence of spontaneous downward tendencies as stated, all the different kinds and degrees of downward progress in the records of animals in this and the preceding paper are purely the results of overstrain of the internal pancreatic function by excess of food.

5. Varying degrees of success and failure in the dietetic control of diabetes are also illustrated. The benefit of the classical treatment by exclusion of preformed carbohydrate and limitation of protein is confirmed, in the prolongation of life and well-being to some extent in nearly all cases and perhaps indefinitely in some of the mildest cases. In the great majority of cases such a therapeutic result is not permanent, and downward progress is finally observed if the observations are continued long enough. In most of these cases life, strength, and assimilation can be preserved for a much longer time by a degree of undernutrition suited to the severity of the diabetes, and accomplished by limitation of fat in the diet. The permanence of such control is supported by the unimpaired or rising assimilation in experiments of 1 to $1\frac{3}{4}$ years duration, but still longer observations would be desirable. Diabetes of great severity is controllable only by correspondingly radical undernutrition. In still more severe cases glycosuria can be abolished only by a degree of undernutrition which entails final death from inanition. In the most severe cases glycosuria cannot be stopped, evidently because the assimilative power is too low to dispose of even the minimum supply of food materials; namely, that derived from the body stores in fasting.

6. A claim of saving every patient, no matter how near death, would be a preposterous one for any remedy in any disease, and the

animal experiments do not support such a claim for diabetes. Also it is unreasonable to expect the actual cure of an organic deficiency by diet, and the diet treatment in animals just as in patients generally represents the sparing rather than the restoration of the weakened function. The basis of the belief in the inherently progressive tendency of severe cases of clinical diabetes is shaken by the exact reproduction of such case histories by diet in animals which are free from spontaneous downward tendencies; but there is still lack of a sufficient number of patients treated on the principle of relief of the total metabolic burden to demonstrate the absence of such inherent progressiveness in human diabetes. As described elsewhere,[8] the principle mentioned has given encouraging results in proportion as it has received actual application in practice. The clinical problem requires the same prolonged careful control of all discoverable symptoms as in animals, and cases too severe for such control, or complicated with infections, violations of diet, etc., are on the same plane as animals in which a similar condition has been produced. Some proportion of cases, especially in young persons, will be found suitable for accurate determination of the question of whether all or most cases of severe diabetes are inevitably progressive and hopeless, and the writer looks forward to publishing such a series. Irrespective of the outcome, the raising of this question is justified by its importance. On the theoretical side, it involves both the general prognosis and the nature of the process underlying diabetes. On the practical side, it is already established that diet is at least the chief cause of downward progress, and it is important to eliminate this cause by avoiding the dietary injuries illustrated in the animal experiments.

[8] Allen, F. M., Stillman, E., and Fitz, R., Total dietary regulation in the treatment of diabetes, Monograph of The Rockefeller Institute for Medical Research, No. 11, New York, 1919.

EXPLANATION OF PLATES.

PLATE 65.

FIG. 1. Dog B2–00. The picture was taken while the animal was diabetic in 1917, but shows approximately the condition existing during the 6 years of laboratory life and still maintained on the restricted protein-fat diet.

FIG. 2. The usual sturdy appearance of Dog B2–01 at her ordinary weight between 13 and 14 kilos.

FIG. 3. Dog B2–01 in July, 1917, when fattened with suet to 16.5 kilos and with hyperglycemia present.

FIG. 4. Dog B2–31 in the state of moderate undernutrition which is indicated by the distinctness of individual vertebræ along the back, and which was effective in abolishing glycosuria for several months. The dog had first become diabetic with $\frac{1}{8} - \frac{1}{5}$ of the pancreas through her gluttony for bread and sugar. Afterward, though undernutrition was maintained, it was not thorough enough. Diets of protein and fat were pushed too close to the verge of tolerance, raising the weight not to normal, but nevertheless higher than the assimilative power could carry. The final result was hopeless diabetes.

FIG. 5. Dog B2–25 in the state of emaciation which had to be maintained for a number of months to control diabetes with only $\frac{1}{12}$ to $\frac{1}{13}$ of the pancreas present. At this weight of 11 kilos the animal remained strong and lively, and required restraint to keep him quiet for photographing. Through hypertrophy of the pancreas remnant it became possible gradually to raise the weight to 17 kilos, or only 1 kilo below normal.

PLATE 66.

FIG. 6. Dog B2–79; control to Dog B2–56. The pancreas remnant was $\frac{1}{8}$, and microscopically showed more inflammation than that of Dog B2–56. The normal weight of 15 kilos was reduced to 11.7, in which condition 200 to 300 gm. of bread were requisite for glycosuria. The animal is shown 8 months after operation, when a diet of 1 kilo of lung had raised the weight to 15.5 kilos, and glycosuria was accordingly present. The dog was kept in good condition and subjected to several other fluctuations of weight and tolerance, till death 17 months after operation.

FIG. 7. Dog B2–80 at a time when glycosuria had been brought on by fattening to 18.2 kilos on a diet of beef lung and suet. The animal was specially valuable because of excellent digestion due to the large remnant ($\frac{1}{4} - \frac{1}{5}$ of the pancreas) with which diabetes occurred. The tolerance was high at weights as low as 13.2 kilos. She was used for various observations during 9 months, and the persistent high fat feeding finally brought on not only glycosuria but fatal acidosis. The dog was as sleek and healthy looking as this when she went into typical coma at a weight of 17.25 kilos. Animals of this sort give the closest reproduction of clinical acidosis.

FIG. 8. Dog C3–27 1 week before death in coma, with intense glycosuria, acidosis, and lipemia present; the dog wags her tail, but she was already nauseated and depressed, and the dry nose is a further sign of ill health. The obesity maintained by the high fat diet is evident.

FIG. 9. Dog C3–56 in the closing period. After the damage of high fat diet, the animal was reduced by undernutrition to a feeble state; but though glycosuria was abolished, hyperglycemia was persistent up to death from cachexia. The similar weakness and deficiency of assimilation of certain human patients under similar conditions are well known.

FIG. 10. Dog C3–86 in moderate undernutrition. The fluctuations of tolerance in parallel with the weight were previously described.[9] The dog was vigorous and lively through $1\frac{3}{4}$ years from the time of operation to death.

FIG. 11. Dog D4–28, $1\frac{1}{4}$ years after an operation which had left $\frac{1}{12}$ of the pancreas. The normal weight was 12 kilos. After prolonged undernutrition, the weight here had been gradually built up to 10.6 kilos. The dog was observed for $1\frac{3}{4}$ years after operation, and remained lively and symptom-free until death from urinary calculus. One feature of the experiment was 50 gm. of bread in the regular ration, showing that a dog with the marked degree of potential diabetes represented by such a small pancreas remnant could by undernutrition be enabled to tolerate such a quantity of carbohydrate for such a length of time without sign of injury.

FIG. 12. Dog D4–52 in Jan., 1918, $\frac{1}{2}$ year after the operation which created mild diabetes. The susceptibility to glycosuria and lowering of assimilation on carbohydrate diet were first demonstrated, and then observations were begun to test whether, with this mild degree of diabetes, a liberal carbohydrate-free diet (with restriction of protein to 500 gm. of beef lung) could be tolerated indefinitely.[10]

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[9] Allen,[7] Chart 8.

[10] Allen,[3] pp. 564–573.

(Allen: Experimental diabetes. I.)

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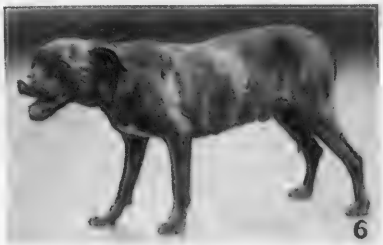

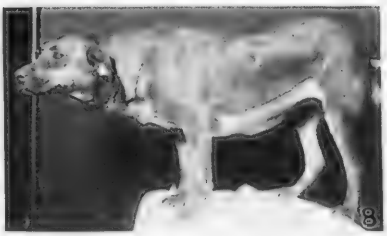

(Allen: Experimental diabetes. I.)

RELATION OF THE PORTAL BLOOD TO LIVER MAINTENANCE.

A Demonstration of Liver Atrophy Conditional on Compensation.

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Plates 67 to 71.

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In the course of observations on the rôle of the liver in blood formation and destruction, we have had occasion to ligate portal branches to the organ. The ensuing changes have been of such striking character as to merit study for their own sake; and the present paper is concerned with them. There already exists, of course, a considerable literature on so obvious a theme. For the moment it may suffice to state in this connection that according to the generally accepted view occlusion of a portal branch to the liver has no effect on the organ save when a grave derangement of the systemic circulation is also present. The complete local parenchymal atrophy that in our experiments regularly followed such occlusion was unforeseen, as was the further observation that the atrophy is conditional, being dependent upon a compensatory hypertrophy of the remainder of the organ.

Method.

The liver of the rabbit is singularly adapted for experiments involving the blood vessels and bile ducts, since it consists of two separate masses, each with its own vessels and ducts. The rabbit may indeed for operative purposes be said to possess two livers. They are of very unequal size, the larger, or main liver, as we shall call it, formed of the left anterior and posterior lobes and the right anterior lobe with the gall bladder, being three times as big as the smaller, or lobe mass, which consists of the right posterior and caudate lobes. The lobe mass contains just enough parenchyma, as Ponfick[1] showed, to suffice for the

[1] Ponfick, E., *Virchows Arch. path. Anat.*, 1889, cxviii, 209; 1890, cxix, 193.

needs of the organism when the main liver is ablated. Through it, under such circumstances, all of the portal stream finds room to pass.

In our experiments the portal trunk to the main liver of the rabbit has been ligated just above the caudate lobe. By such means the whole portal stream is diverted to the lobe mass. The caudate lobe, though a part of the latter by reason of its parenchymal connection with the right posterior lobe, has an added source of venous blood through a small branch arising from the portal trunk at the level of the ligature and frequently compromised by it. To avoid the irregularity thus introduced, the caudate lobe was tied off and cut away as a routine measure. A piece of the tissue was sectioned to determine the condition of the liver.

In ligating the portal trunk great care was taken not to interfere with the main bile duct and hepatic artery. The operation was carried out under ether on rabbits of from 1,400 to 2,300 gm. Closure was done in three layers. Occasionally a fatal necrotic process spread from the ligated caudate stump, but the great majority of the animals recovered without complication and remained in perfect health. They were killed with chloroform at periods of from 12 days to 6 months after the original operation. The liver masses were weighed separately after the blood, as yet unclotted, had flowed away from the severed vessels. This, it was felt, would result in a truer estimate of relative amounts of parenchyma than if the blood were retained by closing the vessels, as was Ponfick's method. According to Ponfick,[1] the normal liver of the rabbit averages 3.56 per cent of the gross body weight, though he prefers to use 4 per cent in calculations. On his estimation the main mass forms 74.7 per cent of the total, the right posterior lobe 19.3 per cent, and the caudate lobe 6 per cent. In fifteen normal rabbits we have obtained an average figure of 3.45 per cent for the liver's proportion of the body weight, with variations ranging from 2.18 per cent to 5.25 per cent. The main mass averaged 72.3 per cent of the total, the ablated caudate 4 per cent, and the right posterior lobe with the caudate stump 23.7 per cent.

Early Changes after Local Portal Occlusion.

The immediate results of diverting the stream are striking.

Within a minute or so the main liver becomes much smaller, of a deeper purple, and flaccid, whereas the isolated lobe, which now receives all the venous blood, is swollen, tense, and of a rather bright red. In animals dying after 1 or 2 days hemorrhages into spleen, stomach, and small intestines are sometimes found, such as Ponfick observed after removal of the main liver; but, as he also noted, these are infrequent in vigorous animals. The acute passive congestion responsible for them is quickly relieved as the stream bed in the lobe mass is widened through the hypertrophy of the latter.

The mass receiving the portal blood begins to hypertrophy within 3 days by cell proliferation within the lobules, as after ablation of the main liver,[2] and by the end of 12 days the tissue has usually more than doubled, and after 15 days may have trebled. Subsequently its bulk increases more slowly, but eventually reaches that of the entire original liver, and usually surpasses it. Concurrent with the hypertrophy is a progressive atrophy of the mass deprived of portal blood (Figs. 1 and 2).

Owing to reduced capillary distension the lobuli fall together to some extent immediately after the ligation, and within the next few days their cells, which now appear crowded, are noted to have grown smaller, especially near the central vein where the blood supply is poorest. If the animal is weak and dies early, a marked local widening of the capillaries may here be noted, with fine, brown pigmentation of the parenchyma. The condition then is identical with that known in human pathology as the "atrophic red infarct of Zahn." In vigorous rabbits the capillary widening is slight, often absent, and the intralobular atrophy alone attracts attention. The liver mass sometimes dwindles within 12 days to about one-half its original bulk (Table I), though usually the change is slower. Its lobes are flabby, wrinkled, purple, and at this particular period their surface is usually mottled with ill defined, slightly raised, pale spots which may be half a centimeter in diameter (Fig. 3). Several lobuli or parts of them are included in such areas. They are well seen only on the capsular surface, are not degenerative in character, become much more prominent when the blood has partially escaped from the tissues, and are probably areas of relative anemia. The general condition of the liver at this time has much in common with that in dogs during the period of adjustment after an Eck fistula.[3,4] But outspoken degenerative changes are rare in the rabbit liver as compared with the dog, though sometimes a moderate, central fatty degeneration may be noted. The tissue when cut is soft, dark purple, and spleen-like; the lobuli are very small and indistinct. Bile ducts, blood vessels, and interlobular connective tissue are all rendered unusually prominent by the dwindling in parenchyma, and, simulating trabeculæ, add to the spleen-like appearance of the tissue.

Microscopically, one finds many small lobuli to a field, and there is a great increase in the number of cells per unit of surface (Fig. 1), so that the nuclei appear crowded. These last, as well as the cytoplasm, have greatly decreased in bulk, though otherwise they appear unchanged. At the periphery of the lobules a few endothelial cells along the capillaries may be somewhat swollen

[2] Ponfick, E., *Virchows Arch. path. Anat.*, 1895, cxxxviii, suppl., 81.

[3] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1917, xlii, 544.

[4] We wish to thank Dr. G. H. Whipple for sections illustrative of the Eck fistula liver.

with granules of a light brown, iron-containing pigment. Such pigmented Kupffer cells become more prominent as time goes on. There is no absolute increase in connective tissue.

Late Changes after Local Portal Occlusion.

The changes up to this point have been partially described by Steenhuis.[5] In his most advanced instance, an animal killed $4\frac{1}{2}$ months after the portal ligation, a further moderate increase in pigmentation and atrophy was noted, but nothing more, a fact difficult to understand save on the assumption that portal collaterals to the main liver had developed, or that the animal was old or in poor condition, all of which factors largely affect the changes. For the atrophy goes further, and quickly too, resulting in a disappearance of all the parenchyma of the main liver, a process sometimes practically completed within 2 months, as we shall show.

Between the 12th and the 40th day after the portal ligation a circulatory readjustment occurs in the dwindling liver mass. Pale spots are no longer seen on its surface, which is of a brighter, more normal red, though tinged with brown. The organ cuts with difficulty, owing to the survival of all its ducts, vessels, and connective tissue, which are brought nearer together by the disappearance of parenchyma; and the tissue disclosed by the knife is more markedly spleen-like, with no trace of a lobular pattern. Histologically, the parenchyma may seem like the normal at first sight, except for the very small size of lobuli and cells, a marked irregularity in the arrangement of the former, and perhaps some irregular capillary widening. Fatty changes are entirely absent, and the atrophy is more evenly distributed. On close scrutiny one perceives here and there parenchymal elements almost without cytoplasm and with small pycnotic nucleus. These scattered cells in the last stages of disappearance are somewhat more numerous toward the center of the lobules. The cell cords in general may be especially atrophic here, and their capillaries wider than at the periphery, additional indications that the central parenchyma suffers most. Pigmented Kupffer cells, distended to a spherical or egg shape, are increased in number, but are found as before only near the periphery of the lobule. The interlobular tissue has nowhere invaded the parenchyma, is absolutely unincreased, and new formed bile ducts are not present. The ducts and vessels, unchanged from their original size, are bent into convolutions as the mass grows smaller, so that their number seems multiplied on cross-section (Fig. 5).

[5] Steenhuis, T. S., Experimenteel en kritisch onderzoek over de gevolgen van poortaderafsluitung, Proefschrift Rijks-Universiteit te Gröningen, 1911.

The period required for complete disappearance of the parenchyma varies with the individual (Table I). When the animal is opened the stomach is found to lie in the concavity of the diaphragm, and only on lifting it away is the insignificant remnant of the main liver discovered, with a gall bladder of normal bigness sessile upon it (Fig. 9). The compensating lobe, of great size, extends far down over the abdomen. On nearer inspection, the original lobes of the main liver are found to be represented by three little, flabby, pinkish brown tags (Fig. 4). Cultures from these are sterile. Their surface is roughened by numerous, close packed and tortuous vessels and ducts. Sometimes red varices stud the surface here and there. The hepatic veins, of almost the normal size, at once attract attention as distended cords, grossly disproportionate to the tissue out of which they spring. The hepatic artery, too, is still large. On section the tissue is extremely tough and shows no parenchyma, but everywhere the gaping mouths of vessels and ducts in a slight matrix of connective tissue. The blood immediately above the ligature on the portal trunk is usually fluid, and thrombi are always absent from the branches of the vein.

Microscopically, a few liver cells can be found even when the changes are most pronounced, for the reason that there exist in practically every case a few minute, collateral venules bringing portal blood to the tissue. The rapidity and completeness of the atrophy is in our experiments proportionate to the number of these little collaterals. Their influence may be directly seen where they enter the liver. For example, in Rabbit 27, killed 118 days after the ligation, 2 minute venules were found coursing to small masses of healthy parenchyma in the midst of the atrophy (Fig. 4).

The final changes have much interest. Little by little the lobular units grow smaller and more irregular in form, so that a central vein, when discoverable, may be far off to one side (Fig. 5). There is still no connective tissue invasion or proliferation, but Glisson's capsule becomes increasingly prominent in the picture, appearing to close in on the lobules and envelop them. As the parenchyma grows less, so do its attendant capillaries disappear, and they never survive when the liver cords are gone. Soon there remain only scattered islands consisting of a few small, but healthy looking liver cords with the characteristic capillaries, set in a matrix of connective tissue (Fig. 7). Here and there, near by, three or four parenchymal cells may perhaps be found as an isolated cord, not infrequently with a capillary along one side; and liver cells separated by the en-

veloping matrix of connective tissue may still be recognized. Many such isolated cells have lost their characteristic ground glass appearance, stain a clear pink with eosin, and may be of blunt spindle shape with a relatively large pycnotic nucleus (Fig. 8). Finally parenchymal elements become rare (Fig. 10). Their situation is sometimes indicated by the irregular zone of Kupffer cells distended with pigment that mark the border of the original lobule. As the atrophy increases such elements become prominent, and rounded nests or aggregations of thirty to forty are not infrequent (Fig. 6). Always they are confined to the region of the original periphery of the lobules and are separated from the parenchyma only by the disappearance of the latter. The total absence of invasive tendencies on the part of the connective tissue could not be more clearly shown than by this fact.

The final tag of ducts, vessels, and connective tissue (Fig. 10), representing 50 to 70 gm. of main liver, weighs, in the absence of coccidiosis, cirrhosis, or other intercurrent proliferative change, only 1 to 1.8 gm., which may perhaps be taken as nearly representing the original weight of the non-parenchymal elements. If a cirrhosis was originally present as shown by the caudate sections, the surviving tissue is of greater bulk.

Conditional Character of the Atrophy.

The liver atrophy in the dog and in man following diversion of the entire portal stream through an Eck fistula is never great. Hence we have questioned whether the complete atrophy observed on local portal diversion in rabbits is inevitable or dependent upon hypertrophy elsewhere. To test the matter hypertrophy has been largely prevented in some animals by tying the bile duct to the lobe mass of the liver after diverting the entire portal stream to it as usual. Under such circumstances the lobe mass undergoes some increase in size through cell proliferation, but combined with this is a continuous, scattered biliary necrosis, and by the end of 12 days a diffuse cirrhosis makes its appearance. After 25 to 30 days the tissue, though still of greater bulk than normal, is indurated and shows microscopically an almost complete replacement with connective tissue. The changes will be more fully described in a later paper. The fact to be emphasized here is that in the absence of hypertrophy of the lobe mass the main liver fails to undergo marked atrophy, although deprived of the portal stream. Such slight atrophy as occurs may be looked upon as inevitable to the circulatory change as such.

The rabbits used were kept under identical conditions and had approximately the same weight. In one series the portal blood was diverted from the main liver and the caudate lobe ablated as usual, while in the other ligation of the bile duct or ducts to the lobe mass was also performed. The local bile stasis had caused no jaundice, and the animals were in good health. The ducts to the posterior lobe, for there may be two or three, lie in an exposed position and can readily be isolated and tied off without damage to their surroundings. From this circumstance, taken with the differing results obtained in the two series of animals, it follows that direct nerve injury can be ruled out as a cause for the complete atrophy after simple portal occlusion. Animals with livers originally abnormal were discarded.

A complicating factor made necessary the early comparison of the series. The progressive cirrhosis occurring when the bile duct from the lobe mass is ligated brings about after a time an obstruction to the portal flow, with chronic passive congestion of the viscera. The spleen enlarges greatly, sometimes becoming cylindrical; and venous collaterals appear rapidly, as a rule preventing marked ascites, though this was once noted. None of the new venous channels had importance for us save such as might enable the portal blood to regain its old stream bed in the main liver; but unfortunately some of this character frequently developed within a few weeks. Usually they followed the course of the veins of Charpy, but sometimes found a way through adhesions. Portal diversion alone not infrequently led to their development in small number. The majority of the rabbits were killed and autopsied prior to their appearance; that is, 12 to 15 days after operation. Such small collaterals to the main liver as were then observed have found place in the general record (Table I). All the animals were killed soon after a feeding.

The weight of the entire liver was somewhat below the normal average in five animals killed 12 days after simple portal occlusion—3.22 per cent of the gross body weight as compared with a normal of 3.45 per cent. Every individual weight, though, was within the normal range of 2.18 to 5.28 per cent. In the five rabbits with local bile stasis added to portal ligation, whereby parenchymal destruction was superimposed upon hypertrophy, the livers weighed more, averaging 3.62 per cent of the gross weight. In the animal with simple atrophy, and its companion with bile stasis, killed after 15 days, the weights were 4.5 and 5.78 per cent of the gross, respectively. The average after longer periods was 3.73 per cent for ten rabbits killed from 21 to 68 days after portal diversion, and 3.69 per cent for three animals with an additional bile stasis, examined after from 21 to 30 days. It would seem that the reparative changes went beyond a mere replacement, as

TABLE I.

Liver Changes Following Diversion of the Portal Stream to the Lobe Mass.

Rabbit No.	Body weight.		Collateral veins to liver.	Liver weight.			Liver's per cent of body weight.			Condition of main liver.	Remarks.
	At operation.	Final weight.		Entire mass.	Main liver.	Lobe mass.	Entire mass.	Main liver.	Lobe mass.		
	Duration of experiment, 12 days.										
	gm.	*gm.*		*gm.*	*gm.*	*gm.*					
1	2,200	2,475	None.	72.4	28.3	44.1	2.93	1.15	1.78	Marked atrophy; many pale spots.	
2*	2,050	2,175	One of 1½ mm.	78.6	43.3	35.3	3.62	1.99	1.63	Little atrophy; no spots.	
3	2,150	2,050	One of ¾ mm.	75.4	39.8	35.6	3.68	1.94	1.74	Marked atrophy; many spots.	
4	2,150	2,050	One of 2¼ mm.	76.6	48.0	28.6	3.74	2.34	1.4	Little atrophy; no spots.	
5	1,900	1,600	One of 3 mm.	46.4	21.7	24.7	2.9	1.36	1.54	Marked atrophy; no spots.	
6	1,925	1,600	Two of 1 mm.	65.2	30.2	32.8	4.07	1.89	2.05	Little atrophy; no spots.	Of total liver weight 2.2 gm. is caudate stump in simple hypertrophy.
7		1,525	One of 1 mm. and several smaller.	48.8	16.8	32.0	3.2	1.1	2.1	Marked atrophy; some spots.	Animal has lost little if any weight.
8	1,850	1,700	None.	55.0	29.9	25.1	3.24	1.76	1.48	Marked atrophy; many spots.	
9	1,550	1,275	One of 1½ mm.	43.1	23.2	19.9	3.38	1.83	1.55	Marked atrophy; many spots.	Animal grew thin after operation.
10	1,750	1,650	One of ½ mm.	56.4	38.3	18.1	3.42	2.32	1.1	Slight atrophy; some spots.	

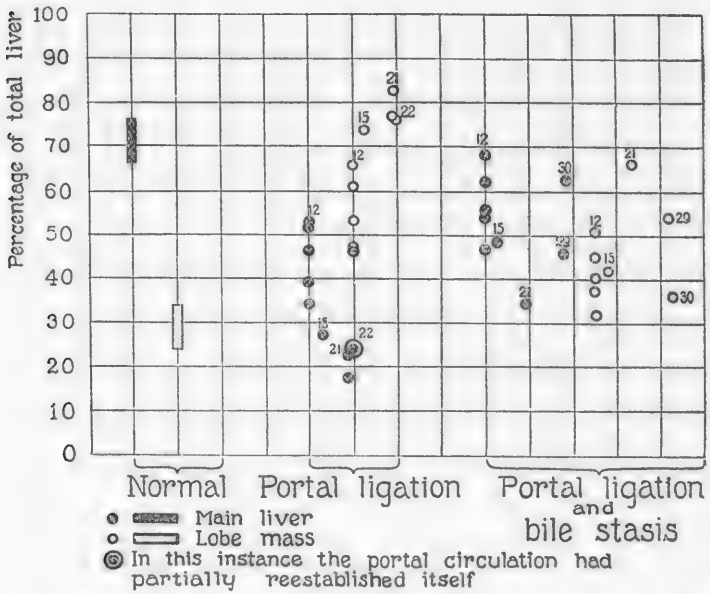
Duration of experiment, 15 days.												
11		1,425	1,525	One of 1 mm.	70.2	18.6	51.6	4.6	1.22	3.38	Marked atrophy; some spots.	
12		**1,425**	**1,400**	**Two of 1 mm.**	**80.9**	**38.9**	**34.2**	**5.78**	**2.78**	**2.44**	**Slight atrophy; no spots.**	**Of total liver weight 7.8 gm. is caudate stump in simple hypertrophy.**
Later periods.												
	days											
13	21	2,300	2,250	Only very fine ones.	73.5	17.0	56.5	3.27	0.76	2.51	Marked atrophy.	At least 2 gm. of main liver consists of coccidial change.
14	29	2,175	2,300	Only very fine ones.	78.5	37.0	41.5	3.42	1.61	1.81	Slight atrophy.	Marked chronic passive congestion. Spleen weight 4.1 gm.
15	30	2,275	2,275	Numerous very fine ones; also one of 1 mm. and of 2 mm.	69.1	44.0	16.4	3.02	1.94	0.72	No atrophy.	Caudate stump of 8.7 gm. in simple hypertrophy gives a by-pass for the blood. No passive congestion.
16	22	1,825	1,900	One of 5 mm.	122.7	30.5	92.2	6.47	1.61	4.86	Moderate atrophy.	Portal flow to main liver almost wholly reestablished.
17	21	1,875	1,375	One of 2 mm.	68.7	22.8	45.9	5.0	1.66	3.34	Moderate atrophy.	Chronic passive congestion; ascites; marked loss of weight.
18	21	1,575	1,750	None (?).	55.4	9.3	46.1	3.17	0.53	2.64	Marked atrophy.	Collaterals not sought for. No passive congestion.
19	21	1,675	1,950	None.	77.5	15.2	62.3	3.98	0.78	2.2	" "	No passive congestion.
20	25			Not noted.	73.5	19.5	54.0				" "	

* The findings in bold faced type relate to animals in which the bile duct of the lobe mass was also ligated.

TABLE I—*Concluded.*

Rabbit No.	Body weight.		Collateral veins to liver.	Liver weight.			Liver's per cent of body weight.			Condition of main liver.	Remarks.
	At operation.	Final weight.		Entire mass.	Main liver.	Lobe mass.	Entire mass.	Main liver.	Lobe mass.		
				Later periods.							
	days	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*				
21	55	2,125	2,550	One of ½ mm.	84.3	5.0	79.3	3.31	0.2	Advanced atrophy.	
22	58	2,025	2,250	Two " 1 "	74.1	10.1	64.0	3.3	0.46	" "	
23	64		2,150	One " 1½ " " " 1 " several hair-like.	59.9	6.7	53.2	2.79	0.31	" "	
24	65		2,050	One hair-like.	70.9	1.8	69.1	3.46	0.09	Practically complete atrophy.	
25	68	2,025	2,050	" of 1 mm.	79.3	4.0	75.3	3.87	0.2	Almost complete atrophy.	
26	104	2,250	2,300	None.	82.4	9.4(!)	73.0	3.58	0.41	Advanced atrophy.	Unusual delay in atrophy.
27	118	1,825	2,375	Two of ½ mm.	99.0	1.4	97.6				Liver weighed with blood retained.
28	185		2,400	" " 1½ " and 1 mm. respectively.	71.1	1.6	69.5	2.96	0.07	Complete atrophy.	

with other tissues. The idea is borne out by the decrease in size of the liver occurring later. In seven animals killed 55 to 185 days after portal diversion the liver averaged only 3.22 per cent of the body weight.



TEXT-FIG. 1. The change in relative proportions of main liver and lobe mass after local portal diversion with and without ligation of the bile duct of the mass receiving the portal stream (see Table I). The range of the normal proportions as observed in thirteen rabbits, is given in the short columns. The caudate lobe had not been ablated in these instances as in the case of the operated individuals. The results in the latter are given in dots, and the number of days elapsing after operation is indicated in small numerals. When there is but one of these above a vertical row of dots it is supposed to apply to all.

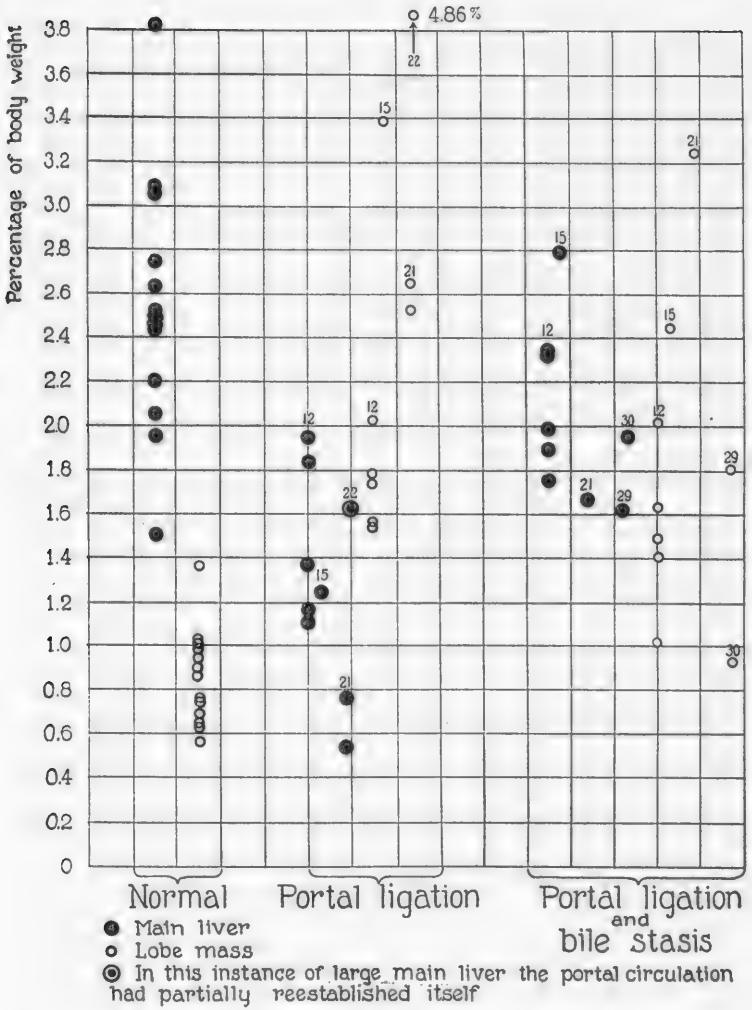
The distribution of the hepatic parenchyma was very different in the two series of animals (Text-fig. 1). Under normal conditions the relative size of the liver masses does not vary much, the larger ranging from 75.7 to 66.3 per cent and the smaller from 24.3 to 33.7 per cent of the whole. After operation not only did the general relations

change but the individual variations in the liver masses became greater. The latter alteration has no interest for us, however. The important point is that the main liver retained far more of its parenchyma after diversion of its portal blood if progressive hypertrophy of the lobe mass was prevented by biliary stasis (Text-fig. 2). The fact is illustrated by differences in both the proportional and actual weights of the liver masses; and the longer the period after operation the greater was the disparity noted, granting the absence of large hepatopetal collaterals. After 4 weeks of portal diversion plus bile stasis the main liver was sometimes three times as big as after but 3 weeks of uncomplicated portal diversion (compare Nos. 14 and 18, Table I, and see also Text-fig. 2).

The importance of portal venules measuring 1 to 2 mm. in diameter for the maintenance of the large mass of liver tissue that remains shortly after operation is negligible, as the general records show (Table I). The animal with the largest collaterals after 12 days, Rabbit 5, the subject of simple portal diversion, had a liver atrophy far greater than occurred in any of the controls with almost no collaterals but with bile stasis. Even at a late period of atrophy, when the liver is greatly shrunken, a collateral of 1 mm. diameter suffices to maintain but a small portion of parenchyma, as we have repeatedly found (Fig. 4). Yet the importance of very large collaterals is not to be gainsaid. It is well seen in the case of Rabbit 16, Table I.

A number of special instances excluded from the table and the text-figures for good cause might be quoted to illustrate the dependence of the pronounced liver atrophy on a compensating hypertrophy. One will suffice.

In a rabbit weighing 1,600 gm., killed 12 days after simple portal diversion, an unexpectedly large main liver was found. It weighed 33.2 gm., representing thus 2.1 per cent of the body weight of the animal, a proportion met with in other animals only when local bile stasis had prevented hypertrophy of the lobe mass. There were no collaterals to explain the condition, but in the lobe mass, which weighed only 22 gm., an atrophy of unknown cause was encountered, affecting at least half of its original tissue content, while the remainder was hypertrophied.



TEXT-FIG. 2. Changes in weight of the main liver and lobe mass, as expressed in percentages of the gross body weight. The number of days elapsing after operation is given in small numerals as in Text-fig. 1.

Functional Activities of the Atrophic Tissue.

The functional activities of an hepatic tissue deprived of portal blood and competing with a hypertrophic parenchyma that receives the entire portal stream have much interest. Asp[6] showed long ago that the immediate effect of a local portal occlusion in the rabbit is to lessen secretion from the affected parenchyma. In our animals the atrophic tissue still secreted bile in good quantity at a period when the liver mass possessing a monopoly of the portal stream was far advanced in compensatory hypertrophy.

The biliary secretion of the rabbit is thin and copious—5 cc. per kilo of animal in 1 hour according to Heidenhain,[7] or 169 gm. per kilo of liver. Krause[8] obtained an average of 115.7 gm. of bile per kilo of animal in 24 hours. The weight of his animals was taken after the intestinal contents had been removed, which entailed a reduction of some 20 per cent from the gross weight. The quantity of rabbit bile varies greatly from hour to hour; the pigments fail to give the ordinary color reactions in a satisfactory way; and neither they nor the other bile constituents have been sufficiently studied to establish a norm. For these reasons we have merely observed the amount and color of the secretion from liver masses in atrophy and submitted it to Pettenkofer's test, while well aware that the latter gives positive results with other substances than bile salts, notably with cholesterol.

Method.

Two operations were necessary, the first to ligate the portal vein to the main liver, and the second, weeks or months later, for the ligation of the bile duct to the atrophic mass and the production of a fistula. The duct was twice tied above the point of entrance of the branch from the compensating lobe mass; the gall bladder was slit at its tip; a fine, rubber-covered catheter was introduced; and this last was sewed to the lip of the abdominal wound in such fashion that the atrophic liver was left in its usual position. The gall bladder and larger bile passages above the ligatures were gently and repeatedly flushed with salt solution injected and withdrawn through the cannula. This was done to wash out all traces of the bile previously reaching the gall bladder from the hypertrophic tissue. The washing never caused even a temporary cessation of the bile flow so far as could be judged from cases in which this was abundant. The cannula was now connected with a thick walled, rubber tube that led to a sterile, flat bottle strapped to the abdomen with adhesive. Through the stopper an air-vent tube was carried around to the animal's back under a many tailed bandage. The

[6] Asp, G., *Arb. physiol. Anstalt Leipzig*, 1873, viii, 124.

[7] Heidenhain, R., in Hermann, L., Handbuch der Physiologie, Leipsic, 1883, v, pt. 1, 252.

[8] Krause, W., Die Anatomie des Kaninchens, Leipsic, 2nd edition, 1884.

bottle was adjusted at such an angle that the bile dropped into it from the end of the tube. The animals tolerated the arrangement well, and sometimes bile collection was continued for a period of 48 hours.

The several experiments in which bile collections were made from main livers in a state of moderate atrophy need not be cited in their entirety. In all an unexpectedly large amount of bile was obtained.

For example, 15 cc. of bright green bile giving a marked Pettenkofer reaction was obtained in 18 hours from an atrophic main liver weighing 19.5 gm., in the presence of a lobe mass of 54 gm. At the time when the bile fistula was produced, 25 days after portal closure, the original ligature on the vein was found to have relaxed, allowing a slight leak to the main liver, and accounting for the slightly delayed atrophy. A second, and completely occluding, ligature was laid on prior to the collection of bile. The latter was secreted at a rate much below the normal, though still considerable—43 cc. per hour per kilo of the atrophic tissue.

More interest attaches to instances in which the compensating mass approximated the whole original liver in size, and presumably in function, since the atrophic main liver had become very small.

Rabbit 24 had on the 65th day after operation a main liver weighing but 1.8 gm. and a compensating mass of 69.1 gm. On the day previous, while the animal was in excellent condition, the main bile duct was ligated and a fistula produced as usual. The ligature was placed above the entrance into the main duct of the branch from the left posterior lobe, and in consequence the secretion from only about two-thirds of the atrophic mass was obtained. At autopsy no portal collaterals to this fraction were found, nor was there the least biliary obstruction. The gall bladder was empty. From it 0.8 cc. of clear, watery fluid had come away in 23½ hours. This had a faint greenish tinge and gave a faint Pettenkofer reaction. The microscope later showed that definite islands of liver parenchyma were present in the tissue furnishing the bile.

A similar duct ligation with the branch from the left posterior lobe excluded was done in Rabbit 23 with a main liver of 6.7 gm., and 53.2 gm. of compensating tissue 64 days after operation. The atrophic tissue from which bile was collected, some two-fifths of the whole main liver, received a portal collateral 1.5 mm. in diameter. Nevertheless, there was secreted from it only 0.9 cc. of bile in 21 hours, though this was medium green in color and gave an outspoken Pettenkofer reaction. The tissue that was drained still consisted predominantly of parenchyma.

Rabbit 21 had a main liver of 5 gm. and a compensating mass of 79.3 gm. on the 55th day after operation. The main duct was ligated successfully, but a blood clot stopped the cannula, so the animal was killed 4 hours after the operation. During this period it had been lively. The stomach was full of food. Only 0.5 cc. of fluid, and this faintly green, was present in the collapsed gall bladder. No obstruction was found to the flow of bile through the ducts; and the

main liver mass was still predominantly parenchymal. A single hepatopetal portal collateral existed, 0.5 mm. in diameter.

The amount of parenchyma present in the atrophic main liver of Rabbit 24 was small. In the other two instances, though, where there was more of it, the amount of bile was far below that called for on calculation, had the conditions been normal, after allowing for 1.8 gm. of scaffolding, ducts, and vessels in the atrophic mass. Furthermore, the secretion in two of the three animals was markedly deficient in pigment, and in one gave but a faint Pettenkofer reaction. Yet there is no doubt that the hepatic tissue, even when extremely atrophic, does manufacture bile of a sort, and is not prevented from so doing by the presence of a compensatory liver mass of very large size. It should be remembered in this connection that the atrophic tissue receives through its large hepatic artery a liberal supply of blood.

Glycogen was sought with Best's carmine stain in three instances of far advanced atrophy. The method is subject to some errors, as Rusk[9] has brought out, yet it seemed preferable to a chemical analysis because of the greatly altered proportion of parenchyma in the tissue. The preparations showed a practically identical amount and distribution of glycogen in the hypertrophic and atrophic parenchyma of the same individual, even in cases as advanced as Nos. 24 and 25 (Table I). Neither the competition of the hypertrophic mass nor its favorable situation on the portal stream was sufficient to deprive the main liver of even relatively little glycogen. But this is scarcely surprising when one considers how widely the substance is distributed in the body, and that dextrose is normally present in the arterial blood. An interesting aspect of the findings is the evidence they give for the belief that such liver parenchyma as survives atrophy to a late period remains in remarkably good condition. For the glycogen content of unhealthy tissues is usually greatly altered.

DISCUSSION.

Conditional Atrophy in the Dog.

Recently one of us, with Dr. Philip D. McMaster, has ligated the portal trunk to the three upper lobes of the liver in a number of dogs. Changes ensued much more slowly than in the rabbit and are not yet

[9] Rusk, G. Y., *Univ. California Pub. Path.*, 1912, ii, 83.

complete, after 3 months, but the tissue deprived of portal blood has diminished to less than one-third of its original bulk through a simple atrophy, with a corresponding hypertrophy elsewhere.

Review of the Literature.

Previous work on the result of local portal occlusion has been well summarized by Winternitz,[10] who himself treats of the early changes in human livers. Though some observers, Solowieff[11] and others, have claimed that the occlusion of portal branches leads to cirrhosis, and Ehrhardt[12] stated that a moderate atrophy without cirrhosis ensues, the prevailing view, long since crystallized, is that no liver changes occur either in man or the laboratory animals unless pressure is abnormally low in the hepatic artery or high in the vena cava as the result of a disturbed systemic circulation. Under such contributing circumstances one finds within a few days the so called red infarct of Zahn in the region deprived of portal blood. Here the lobular capillaries are much distended, presumably from a venous stasis, and the liver cords somewhat atrophied. In the gross the liver portion is dark red and slightly sunken. The late changes have not been described, according to Winternitz, for the reason that such diseases as produce embolus or thrombosis in the portal system almost always end fatally within a brief period.

It is interesting to note here and there in the literature isolated statements that confirm our findings, and like them indicate that the prevailing view as just given is erroneous. Thus Frerichs[13] stated in 1858 on the basis of his own observations that local portal occlusion leads to parenchymal atrophy with liver scarring. According to Ehrhardt,[12] Nauwerck saw a case in which the left lobe of the liver was diminished to the size of the fist, with compensatory hypertrophy of the right lobe, as a result of long standing occlusion of the left portal branch. Ehrhardt himself produced a moderate atrophy with compensatory hypertrophy by local portal ligation in cats, but he did not follow the changes long.

As already mentioned, most authors state that the development of a red infarct of Zahn after local portal occlusion is conditional upon a general circulatory disturbance. The fact that Zahn[14] himself produced typical red infarcts by injecting mercury into a mesenteric vein of otherwise healthy dogs seems to have been forgotten, as has also his view that the condition is an atrophy from inactivity owing to the lack of portal blood, combined with a pressure atrophy

[10] Winternitz, M. C., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 396.

[11] Solowieff, A., *Virchows Arch. path. Anat.*, 1875, lxii, 195.

[12] Ehrhardt, O., *Verhandl. deutsch. Ges. Chir., 31 Kong.*, 1902, xxxi, 544.

[13] Frerichs, F. T., Klinik der Leberkrankheiten, Brunswick, 1858 (Sydenham Society's translation, A clinical treatise on diseases of the liver, London, 1861).

[14] Zahn, F. W., *Verhandl. Ges. deutsch. Naturforsch. u. Aerzte, 69 Versamml.*, 1897–98, ii, pt. 2, 10.

from stasis as the result of retrograde pressure in the hepatic veins. The drawing given by Chiari[15] shows a histological condition identical with that present in our rabbits 12 to 15 days after the ligation. There can be no doubt that a general circulatory derangement renders the atrophy more prominent, as was the case in some of our animals that fell sick. The widening toward the central vein of the lobular capillaries, when marked, is doubtles often the result of retrograde pressure; but it occurred in moderate degree in some of our well conditioned rabbits and would seem then to have been merely the consequence of rapid atrophy of the liver cords. That there may occur complete parenchymal atrophy which is conditional on hypertrophy of the remaining parenchyma has not been realized heretofore.

Since the completion of our work the paper of Steenhuis[5] has come to attention. It has attracted little notice among pathologists, owing perhaps to the fact that its author laid stress rather upon the surgical implications of his findings than upon the pathological. But Steenhuis ligated the portal trunk to the main liver of the rabbit, just as we have done, and observed an atrophy of medium grade thereafter, with the development of pigmented Kupffer cells. He did not follow the changes to even approximate completion, since in his most advanced instance a considerable bulk of liver tissue still remained, as the pictures and description clearly show. He noted the influence of portal collaterals to check the atrophy and drew the conclusion, since proved erroneous, that a direct portal stream is essential to liver survival.

Physiological Considerations.

Several reasons can be suggested for the changes which follow a local diversion of the portal stream. Among them are the following:

(*a*) *Direct Influence of the Altered Circulation.*—By the ligation of its portal trunk the main liver is supplied solely with arterial blood, which latter may be so unsuited to the liver cells that they can survive and function only in the absence of competition, as under the circumstances of an Eck fistula. The high oxygen content of the blood can scarcely be invoked as a cause of the hypothetical unfitness, since the atrophy is least at the periphery of the lobules where oxygenation is greatest.

(*b*) *Altered Functional Opportunities.*—By local portal obstruction one portion of liver tissue is deprived of its normal opportunity to obtain many substances and must compete with another receiving them in undue quantity. Functional atrophy and hypertrophy should follow as a matter of course. The extent and rapidity of the

[15] Chiari, H., *Z. Heilk.*, 1898, xix, 475.

changes alone are surprising. The hypertrophy goes on almost, perhaps quite, as rapidly as if the tissue deprived of portal blood had been ablated.

Few rabbits survive the abrupt removal of the main liver. Ponfick's instances do not enable one to judge when the compensating hypertrophy was complete. Von Meister[16] states that the right posterior lobe and caudate attain the weight of the whole liver in from 45 to 60 days. But this weight he puts at only 2.91 per cent of the gross body weight, whereas 3.45 per cent is nearer the truth and has been the basis of our calculations. In one of our cases such a proportion to the body weight was actually attained by the hypertrophied mass within 65 days and in another within 68, while the functional adequacy of the tissue was attested by complete atrophy of the main liver in one instance and approximately complete atrophy in the other. These results become more striking when one considers that our animals were adults weighing 2,000 gm., whereas von Meister's were young and of 900 to 1,400 gm., that is, far more favorable to hypertrophy, as he showed; and when the further fact is added that the caudate lobes were cut away in our cases so that there was less tissue capable of hypertrophy. Nasse[17] found that 4 months was required for the disappearance of the main liver mass of the rabbit after ligation of its bile duct.

If the rate of the hypertrophy is approximately the same after local portal deprivation as after local ablation, this might mean either that the tissue deprived of portal blood is useless to the organism or that hypertrophy goes on irrespective of its activities. The pros and cons cannot be profitably discussed, but both alternatives entail the assumption that the liver is wholly a portal organ, finding its reason for being in the substances carried to it on the portal blood and in them only. The biliary activity of the atrophic tissue does not constitute evidence against such a view, even granting that the substances from which bile pigment is produced come to the liver normally on the portal blood alone—an assumption yet to be proved. The small hepatic mass which receives the entire portal blood after local diversion of the stream must be thought of as unable to cope with its functional opportunities for some time, so that much material for liver activity passes through into the general circulation and reaches the atrophic competing mass. Later, as the compensating tissue attains the size and functional power of the whole original liver, less of the portal material may be supposed to escape through it. Yet

[16] von Meister, V., *Beitr. path. Anat. u. allg. Path.*, 1894, xv, 1.
[17] Nasse, *Verhandl. deutsch. Ges. Chir.*, 1894, xxiii, 525.

that a portion does pass through the normal liver has been shown by Van Slyke[18] for the amino-acids. It is perhaps a similar passage of material that enables a liver remnant wholly deprived of portal blood and advanced in atrophy to continue the formation of bile pigment, albeit in reduced quantity.

(*c*) *Food Deprivation.*—Though the main liver mass of our animals undoubtedly received relatively little of some at least of the substances upon which it normally acts, this need not have been the essential cause of its atrophy. Perhaps a special food is essential to liver maintenance. Whether this comes from a systemic or portal source, the tissue receiving the whole portal stream would have the best opportunity at it, and, possessing the superior powers of a growing, healthy tissue, would gradually increase its rival's deprivation. But were the source of the hypothetical food substance systemic, not portal, one would expect atrophy and hypertrophy to go on more slowly than is the actual case and perhaps not to reach completion. The question whether food substances can be distinguished from those utilized in function need not be entered upon.

Much of the foregoing incomplete analysis is only warranted in as far as it illustrates the vital importance for the liver tissue of a position on the portal stream. A situation there is not obligate, it is true. For the liver deprived of the direct portal stream by an Eck fistula still survives,—though perhaps only because it still receives the portal substances, at one remove, so to speak, by way of the systemic circulation.

Influence on Liver Development.

Toldt and Zuckerkandl[19] demonstrated in 1876 that the normal human liver undergoes notable changes in shape during the period from birth to adult life. In some portions of the organ atrophy occurs, while in others there is hypertrophy. The atrophy seems to be identical with that after portal diversion, while, when it is complete, as not infrequently happens, there are left behind the same large corded ducts and blood vessels. Toldt and Zuckerkandl attribute the changes to pressure from the surrounding organs and adduce

[18] Van Slyke, D. D., *Arch. Int. Med.*, 1917, xix, 56.

[19] Toldt and Zuckerkandl, *Sitzungsber. k. Akad. Wissensch. Wien., 3te Abt.*, 1876, lxxii, 241.

reasons for their belief. We would go a step further and suggest that the transmitted pressure may produce its effect in some instances through local alterations in the portal stream.

Mall,[20] who confirms the findings of Toldt and Zuckerkandl, points out in another connection that the distribution of the portal blood to the hepatic parenchyma is in general remarkably even, as can be demonstrated by injection methods. No hepatic region is specially favored. In the light of our observations the necessity for this is clear. For any enduring local irregularity in the portal flow will result in a shift of parenchyma. One of the commonest shifts observed by Toldt and Zuckerkandl entailed a complete atrophy of the left lobe. Herringham[21] found ten such cases in 3,000 autopsies. A reason for this is not far to seek, nor for the rarity of atrophy of the right lobe. In man the right branch of the portal vein is extremely short and thick, breaking up almost at once into many lesser vessels; whereas the left branch courses for a long distance through the parenchyma as a single slender trunk, much exposed to transmitted pressure.

Bearing on Liver Lesions.

Pathologists have long recognized that liver destruction frequently induces a local compensatory hypertrophy. From our observations it is evident that there exists, conversely, a type of destruction dependent upon compensatory hypertrophy. The knowledge should aid in an understanding of certain chronic liver lesions. The advanced local atrophy sometimes occurring in livers containing an echinococcus cyst, a gumma, slow growing tumor, or other limited process may well be the result of pressure upon portal radicles. In such instances there is present elsewhere in the organ an abundance of parenchyma capable of compensatory proliferation. The opportunities for marked changes are far less favorable in the atrophic cirrhosis of Laennec. The irregular stenosis and occlusion of portal branches which characterize the disease fail to lead to a complete atrophy of large liver portions because the parenchyma which under ordinary circumstances would proliferate in compensation is prevented from so

[20] Mall, F. P., *Am. J. Anat.*, 1906, v, 227.

[21] Herringham, W. P., *St. Bartholomew's Hosp. Rep. 1905*, 1906, xli, 15.

doing by a confining connective tissue. However, numerous small areas of partial hypertrophy and atrophy may and do exist.[22] In syphilitic livers with sharply localized scarring no such impediment is present; and we would suggest that local portal obstruction is a prime cause for the extreme atrophy and hypertrophy which in such cases frequently lead to great hepatic distortion. According to Sternberg[23] a whole lobe of the syphilitic hepar lobatum may be reduced to a connective tissue appendage.

The disappearance of large masses of liver tissue without the least connective tissue replacement may take place in the very old. According to MacCallum[24] whole layers of parenchyma may disappear, . . . "on the surface of the organ blood-vessels, bile-ducts, and the fibrous skeleton of the liver lie exposed." Not infrequently the atrophy is one of deprivation, and identical in its essentials with that resulting from local portal occlusion.

SUMMARY.

The occlusion of portal branches to a part of the liver of the rabbit leads to a progressive and ultimately complete atrophy of the parenchyma in the region deprived of portal blood, and to hypertrophy of the rest of the hepatic tissue which receives such blood in excess. Three-fourths of the liver may thus be reduced to a fibrous tag within 2 months, while the remaining fourth attains the bulk of the entire original organ. The atrophy is simple, unaccompanied by obvious degenerative changes or by any connective tissue replacement. More important, it is conditional in nature, failing to progress when the bile duct from the proliferating tissue is ligated and its hypertrophy checked in this way.

There are indications in the literature that an atrophy conditional on hypertrophy, such as is here described, occurs in man after local portal occlusion. And some experiments in our laboratory, not yet completed, show definitely its occurrence in the dog. The changes take place slowly in the canine liver. After 3 months the tissue de-

[22] MacCallum, W. G., *J. Am. Med. Assn.*, 1904, xliii, 649.

[23] Sternberg, C., in Aschoff, L., Pathologische Anatomie, Jena, 1911, ii, 855.

[24] MacCallum, W. G., Text-book of pathology, Philadelphia and New York, 2nd edition, 1914, 60.

prived of portal blood has diminished to about one-third of its original bulk. The conditional character of the atrophy is proven by its failure to occur to any similar degree in the absence of a compensating parenchyma, as when the portal stream is diverted from the whole liver by way of an Eck fistula.

Is the atrophy functional? If so, its completeness would indicate that the liver has no essential activity—none on which its maintenance depends—that it is not intimately connected with substances derived from organs drained by the portal system. Observations on the rate of hypertrophy after local diversion of the portal stream and on the character of the bile secreted by the atrophic tissue may be taken to favor such a view. The hypertrophy is nearly, perhaps quite, as rapid as if the tissue deprived of portal blood had been removed from the body. The bile secreted from a liver mass far advanced in atrophy and competing with a large bulk of parenchyma that receives the entire portal stream is almost colorless and may give but a weak Pettenkofer reaction. Glycogen, on the other hand, is present in the atrophic cells in approximately the same amount and distribution as in the hypertrophic liver tissue of the same animal.

The fact that a parenchymal shift follows local disturbances in the portal stream has a bearing on the cause of certain alterations in the shape of the normal liver that have been loosely attributed heretofore to pressure from the surrounding organs. It also has some interest in connection with pathological changes. Liver hypertrophy dependent on a preceding destruction has long been known to pathologists. Now a type of destruction dependent on compensatory hypertrophy must also be reckoned with. The occurrence of changes of the latter character will explain certain of the lesions observed in diseases that involve a disturbance of the portal flow to portions of the liver substance.

EXPLANATION OF PLATES.

PLATE 67.

FIGS. 1 and 2. Rabbit 7, Table I. Hepatic atrophy and hypertrophy, respectively, 12 days after diversion of the portal stream from the main liver. Three lobules of the main liver are barely equal in size to one of the hypertrophic lobe mass. The cells in atrophy are smaller and appear crowded together. Hematoxylin and eosin.

PLATE 68.

FIG. 3. Rabbit 9, Table I. Pale spots on the surface of a main liver 12 days after the ligation of its portal trunk. To the right is seen the corresponding hypertrophic lobe mass.

FIG. 4. Rabbit 27, Table I; 118 days. The effect of portal collaterals. The main liver is in complete atrophy save for a small, button-like area of healthy looking parenchyma, which receives at its center a portal venule from the lesser curvature of the stomach. Fig. 9 is a photograph of the entire specimen, and Fig. 10 shows the microscopic findings in the main liver.

PLATE 69.

FIG. 5. Rabbit 21, Table I; 55 days. Advanced atrophy of the main liver. The parenchyma is greatly diminished in amount, and the surviving lobules are extremely irregular. Bile ducts and blood vessels are prominent and numerous, and the intralobular capillaries are much widened in this special instance, but the lobules themselves are uninvaded by connective tissue which, however, is definitely increased. The increase was present prior to operation, as the liver fragment taken at the time shows. The dark rounded masses here and there are Kupffer cells distended with pigment. Hematoxylin and eosin.

FIG. 6. Rabbit 25, Table I; 68 days. Another instance of advanced atrophy of the main liver, but with pigmented Kupffer cells in unusual abundance. The peculiar appearance of the liver cells is due to the fixative. The great number of bile ducts relative to parenchyma should be noted, as also the characteristic absence of any increase in connective tissue, save for a slight thickening about the ducts, that was present prior to operation. Hematoxylin and eosin.

PLATE 70.

FIG. 7. Rabbit 24, Table I; 65 days. A late stage of parenchymal disappearance. Two small islands of liver cords with characteristic capillaries can be discerned. Many ovoid, pigmented Kupffer cells are present here and there. Methylene blue and eosin.

FIG. 8. A highly magnified parenchymal island from the same specimen. Near it are individual liver cords and cells isolated by the enveloping connective tissue. To the right and left lie Kupffer cells distended with pigment. Methylene blue and eosin.

PLATE 71.

FIG. 9. Rabbit 27, Table I; 118 days. Practically complete atrophy of the main liver, with compensatory hypertrophy of the lobe mass. In Fig. 4 a nearer view of the main liver is given.

FIG. 10. Condition of the main liver in the same rabbit. Save for an occasional cell, which cannot be discerned in the picture, the parenchyma is entirely gone. There remain arteries, veins, and bile ducts in a slight matrix of connective tissue, with some aggregations of round cells. Eosin and methylene blue.

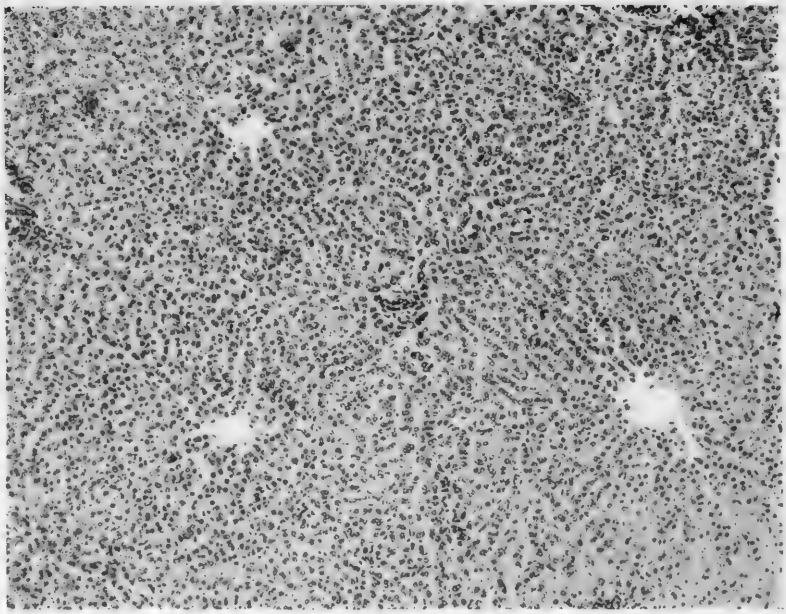


FIG. 1.

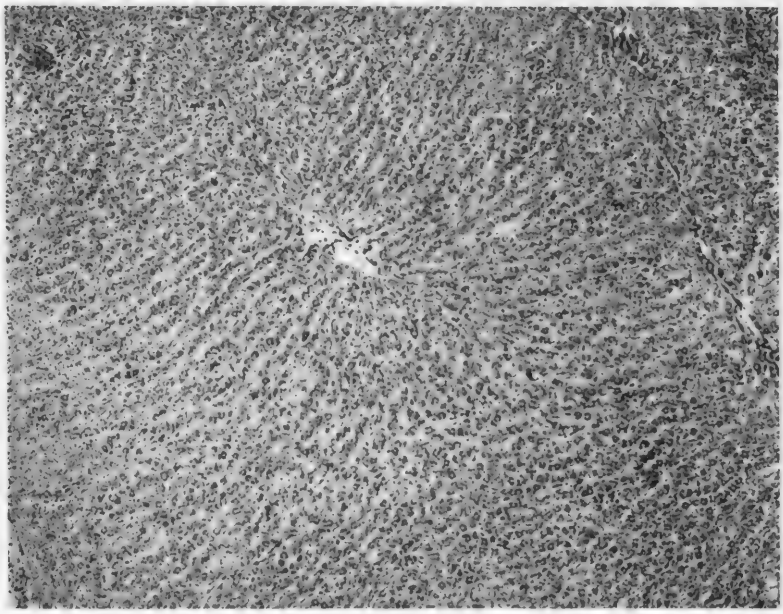


FIG. 2

(Rous and Larimore: Portal blood and liver maintenance.)

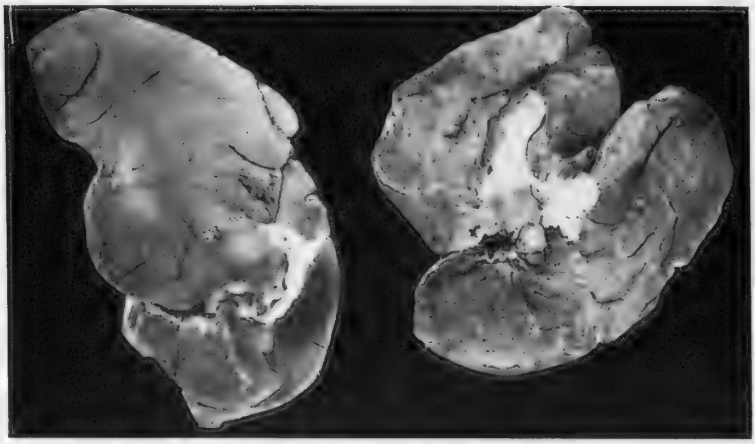


FIG. 3.

FIG. 4.

(Rous and Larimore: Portal blood and liver maintenance.)

FIG. 5

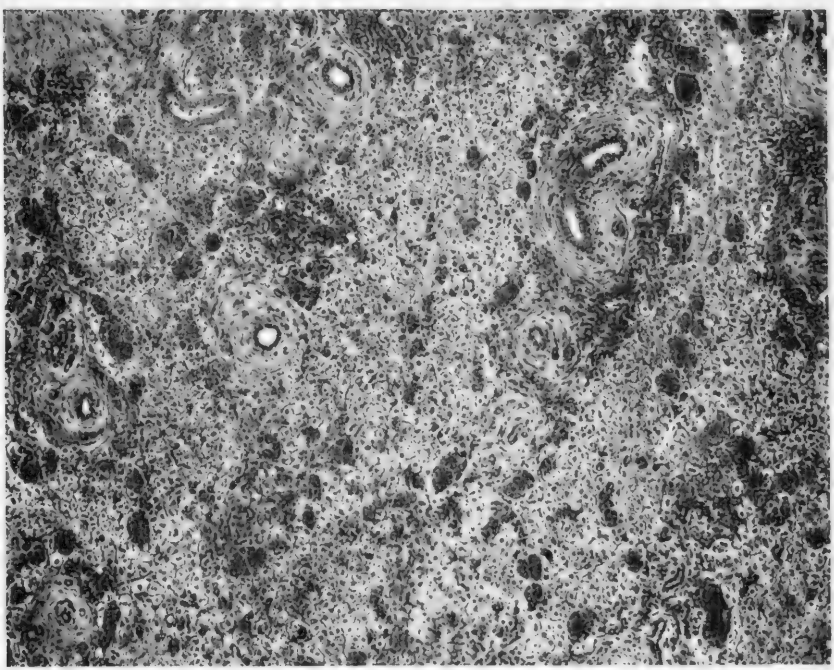


FIG. 6.

(Rous and Larimore: Portal blood and liver maintenance.)

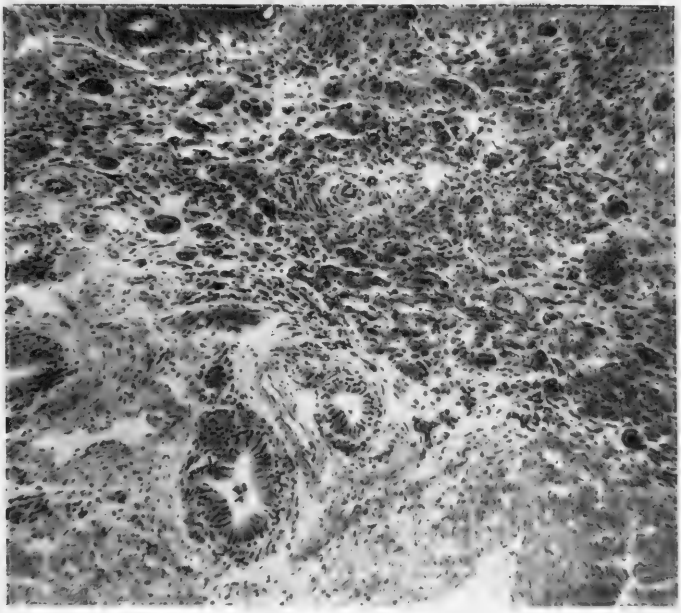


FIG. 7.

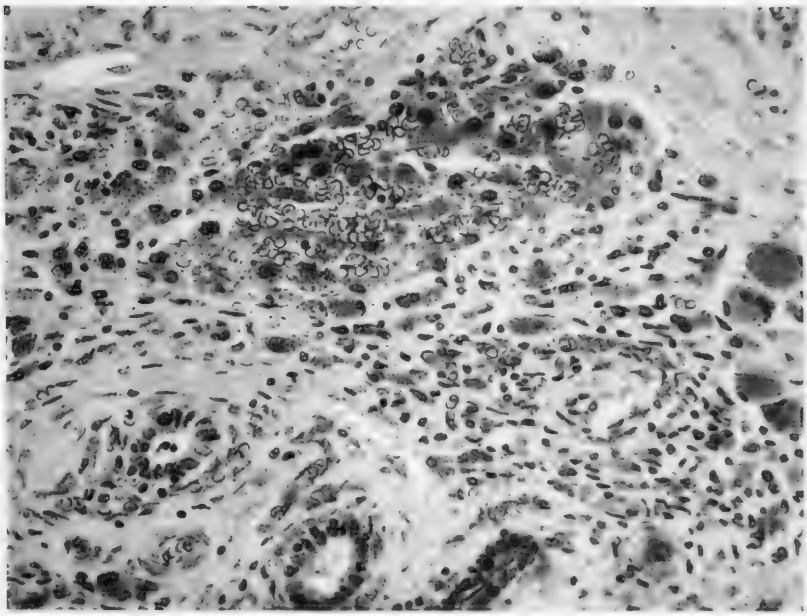


FIG. 8.

(Rous and Larimore: Portal blood and liver maintenance.)

FIG. 9.

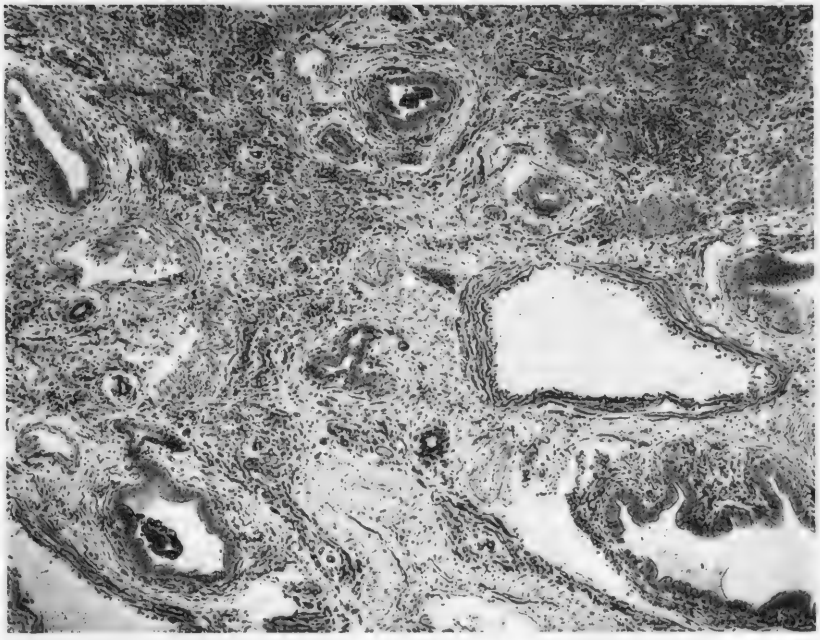


FIG. 10.

(Rous and Larimore: Portal blood and liver maintenance.)

EPIDEMIOLOGY OF BLACKHEAD IN TURKEYS UNDER APPROXIMATELY NATURAL CONDITIONS.

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The field experiments to be described in this paper are continuations of those made during the warm season of 1916 and published the following year.[1] The results of this earlier work indicated that the exposure of young incubator turkeys to older birds from infected flocks on infected ground produced disease within 2 weeks; exposure to older infected birds on fresh territory also produced disease, but after a somewhat longer period, and exposure to manifestly diseased young stock did not yield positive results. The last mentioned outcome might lead to various inferences, such as the elimination of unripe stages during active disease, the existence of an intermediate host, the need for a period of incubation in the soil, and so on.

The work of the years 1917, 1918, and 1919 was designed to repeat that of 1916, while furnishing ample material for the prosecution of other lines of work. However, as will be shown, the exposure of young turkeys to infected stock yielded cases of disease at irregular and uncertain intervals, so that intensive work on more specific problems had to be postponed until a method of producing blackhead with certainty and within a definite period of time could be worked out.

The experiments were conducted as heretofore with turkeys raised in the incubator and brooder and subsequently confined in outdoor enclosures of limited extent, varying in area from about 300 to 10,000 square feet. Each enclosure contained a small house in which the turkeys were kept at night. No attempt was made to cover these enclosures so as to exclude rodents, sparrows, and migrating birds.

[1] Smith, T., *J. Exp. Med.*, 1917, xxv, 405.

For it was considered of importance to determine if possible whether carriers, other than turkeys, exist. All experiments unless otherwise stated were conducted with turkeys raised in the incubator and brooder.

The term "blackhead" which has found its way into common use as referring to the disease due to the invasion of the walls of ceca and liver with a specific protozoan parasite, *Amœba meleagridis*, is used in these pages for convenience's sake.

Experiments of 1917.

Three separate experiments were made in three outdoor enclosures. One was designed to test the infectiousness of young diseased turkeys, the other that of poultry from large flocks. The third contained controls. The turkeys used were hatched in three lots on dates beginning May 23 and ending June 16.

Experiment 1.—To test the infectiousness of young diseased turkeys, the latter were obtained from a nearby farm. Of a lot of eleven hatched by a hen and a turkey, three had died and the diagnosis had been confirmed on one by autopsy. Aug. 20. Four of the remainder, 5 to 6 weeks old, were introduced into the experimental enclosure containing four healthy turkeys. Aug. 25. One infected turkey died of blackhead. Aug. 26. A second died of the same disease. Aug. 29. The third died of coccidiosis. Blackhead lesions were not detected. Aug. 30. The fourth died of blackhead complicated with coccidiosis. The incubator turkeys were thus exposed for 10 days to both blackhead and coccidiosis in four living birds, and after the death of the latter to whatever soil infection resulted, until Nov. 9, a period of more than 2 months. No deaths occurred and no illness was observed. Nov. 9. One of the four was killed and found free from any lesions. The droppings of these birds had been examined in the course of the experiment and at the close the droppings of all were sedimented and examined for coccidia and ova but none were found. It should be stated that in the infected birds there were found in addition to the coccidia, specimens of *Heterakis papillosa*.

Experiment 2.—Aug. 20. To test the effect of exposure to chickens, four White Leghorns from four different flocks, three from New Jersey and one from Massachusetts, were placed in an outdoor enclosure and four turkeys placed with them. Turkey 175 was hatched on May 23, Nos. 178 and 185 on May 29, and No. 190 on June 16. No. 175 was first noticed to be sick on Sept. 28, 39 days after the beginning of the experiment. It was chloroformed and characteristic lesions of blackhead were found in ceca and liver. One individual of *Heterakis papillosa* was found in one cecum. The other three turkeys remained well. No. 185 was killed on Nov. 8, over 2½ months after the beginning of the exposure, and was

found free from any lesions or scars indicative of an attack. Four individuals of *Heterakis papillosa* were present. The two remaining turkeys were not killed but the feces were collected, washed, and sedimented. Neither ova nor coccidia were found.

Experiment 3.—Control enclosure. Eight incubator turkeys left after taking out those used in Experiments 1 and 2 were kept in an outdoor enclosure during the entire summer (Nos. 176, 179, 180, 182, 184, and 186 to 188). Aug. 25. No. 176 died. The feet of this turkey had been deformed and it was finally unable to walk. No blackhead lesions were present. Oocysts of coccidia were found in the ceca. No. 186 became sick on Sept. 14 and was killed on Sept. 21. Blackhead lesions were present in ceca and liver. No. 179 was killed Nov. 6 and No. 180 on Nov. 7, to determine if the birds had passed through a mild attack. No lesions or scars were present in either bird. Worms, ova, and coccidia were absent. The feces of all but No. 176 had been examined during the last week in Aug. and coccidia found in Nos. 184 and 187.

This group of experiments is significant from several points of view. It shows that blackhead may appear in an isolated group of turkeys and suggests that birds other than turkeys or hens may be carriers of the parasite. The disease appeared first in the control group on September 14 and 2 weeks later in the group with the chickens. It is highly probable that the same outside agencies brought the disease to both groups and that the chickens were not responsible. Singularly, the turkeys associated with three fatal cases of blackhead and one of coccidiosis remained healthy during the entire season, thus confirming earlier work that young diseased birds do not transmit blackhead. In accordance with this fact is the low morbidity in Experiments 2 and 3. Although each group was exposed to a case of blackhead occurring among its own numbers in September, yet no further illness occurred during the remainder of the season.

Experiment 4.—Towards the close of 1917 some individuals of the three groups (Experiments 1, 2, and 3) were brought together in the enclosure of Experiment 1 with a male, No. 125, which had been raised in the incubator in 1916 and exposed to disease during the summer of that year. This bird was therefore 1½ years old. A second group was formed on the grounds recently occupied in Experiment 3 as a control. Table I summarizes the condition when the groups were broken up on June 14, 1918. One in each group had been killed in Jan. and found normal. All in the control group were well. In the other group one died of blackhead on May 15 and two were ill about the same time, but recovered. They may have

passed through an attack of blackhead. One had been injured by the male and this may have accounted for the illness in one case. Both were still alive at the beginning of 1920. Several interpretations may be put on the outcome. Blackhead may have been transmitted by the old infected male (see Experiment 6), or by some individuals from Experiments 1, 2, and 3 which had become carriers, or by some outside agency.

TABLE I.

Exposed in enclosure of Experiment 1, Nov. 9, 1917, to Male No. 125, until June 14, 1918.

No. of turkey and group to which it had belonged.	Subsequent history.
181 (1)	June 14, 1918. Well.
189 (1)	Jan. 28, 1918. Killed. Normal. *Heterakis papillosa* in ceca.
184 (3)	May 23, 1918. Sick. Recovered.
188 (3)	" 15, 1918. Died. Blackhead.
178 (2)	" 20, 1918. Sick. Recovered.
Controls (in Enclosure 3).	
182 (3)	June 14, 1918. Well.
187 (3)	" 14, 1918. "
190 (2)	" 14, 1918. "
183 (1)	Jan. 30, 1918. Killed. Normal. *Heterakis papillosa* in ceca.

Experiments of 1918.

Experiment 5.—This experiment was performed to test the relative infectiousness of older turkeys which had been exposed in the preceding year.

The flock to which young turkeys were to be exposed consisted of one from Experiment 1, two from Experiment 2, and three from Experiment 3 of 1917. With this flock the male referred to in the preceding experiment, No. 125, remained for a short time. They had occupied the same enclosure since the preceding year. The infectious character of the environment was demonstrated by the fatal case of blackhead of May 15, referred to in Experiment 4.

To imitate the usual conditions, one of these turkeys was made to incubate ten eggs. Six hatched on May 22. One young was killed in the nest, leaving five. One died on June 13, probably of general weakness. No infection could be demonstrated. On this day the remaining four were allowed to run with the older turkeys. All died of blackhead, one on July 22, one on Aug. 11, one on Aug. 13, and the last one on Sept. 8. After the death of the young stock, three additional young incubator turkeys were exposed on Sept. 17. Two of these contracted the disease on Oct. 2 and Nov. 12, respectively. The third, killed on

Nov. 25, 1919, showed some suspicious smooth areas in the ceca, but no definite lesions were found.

Two turkeys were introduced into this enclosure as late as Nov. 29. One of these contracted blackhead in Jan., 1919. The other remained well.

To test the susceptibility of chickens, four, hatched in an incubator and running only with incubator turkeys, were placed in this enclosure on Sept. 3. They

TABLE II.

Turkey No.	Source.	Exposure.	Result.
254	Hatched by No. 181 on May 22.	Exposed to mother turkey in coop until June 13, then to the old flock.	July 22. Died of blackhead, after 39 days.
255	Hatched by No. 181 on May 22.	" "	Aug. 11. Died of blackhead, after 59 days.
256	Hatched by No. 181 on May 22.	" "	Aug. 13. Died of blackhead, after 61 days.
257	Hatched by No. 181 on May 22.	" "	Sept. 8. Died of blackhead, after 87 days.
242	Hatched by No. 181 on May 22.	" "	June 13. Died, general weakness.
	Hatched by No. 181 on May 22.		Killed by accident in the nest.
264	Hatched in incubator.	Exposed on Sept. 17.	Oct. 5. Chloroformed. Affected with blackhead.
263	Hatched in incubator.	" " " 17.	Nov. 25, 1919. Killed. Normal except for a few possible scars in ceca.
262	Hatched in incubator.	" " " 17.	Nov. 19. Chloroformed. Affected with blackhead.
265	Hatched in incubator.	" " Nov. 29.	Jan. 21, 1919. Chloroformed. Affected with blackhead.
248	Hatched in incubator.	" " " 29.	Remained well.
Four chickens.	Hatched in incubator.	" " Sept. 3.	All remained well.

remained well and were killed, two 65 days and two 80 days after the beginning of the exposure. No lesions were found. Table II summarizes the results of this experiment.

Probably the most significant feature of this experiment is the fact that sooner or later nearly all the exposed died, but the period between the beginning of exposure and actual disease was very variable.

Experiment 6.—June 14. Four incubator turkeys were penned on new ground with the old male, No. 125, referred to in Experiments 4 and 5. July 9. Four additional young turkeys were placed in the same enclosure. Aug. 23. The old male was killed and found normal. Aug. 28. Experiment closed. None of the exposed showed signs of blackhead.

Experiment 7.—In this experiment young turkeys were penned with chickens from two sources. From each source a chicken had been found affected with blackhead. The first lot of chickens was penned with three healthy turkeys, beginning July 5. Dec. 20. Two of the turkeys were killed and found normal. The third was reserved.

Sept. 18. The second lot of chickens was penned with two turkeys. After an exposure of 69 days, one turkey was killed and found normal. The other was reserved for breeding.

TABLE III.

Lot No.	Date of hatching.	No. in each hatch.	Beginning of outdoor life.	Cases of blackhead.	No. running together at the end of.
	1918		*1918*		
1	Apr. 24	10	May 9	None.	June, 48.
2	May 12	25	" 27	Nov. 27. (One.)	July, 39.
4	" 26	24	June 3	Dec. 20. (")	Aug., 37.
5	" 28	(*a*) 10	" 1	June 22, Sept. 21, Nov. 6,	Sept., 31.
		(*b*) 8	" 3	12, Dec. 14. (Five.)	Oct., 30.
6	June 13	15	" 15	Oct. 26. (One.)	Nov., 15.

Experiment 8.—A study was made of the epidemiology of a large flock not exposed experimentally to disease. In the spring of 1918 the possibility that the eggs might be infertile led to the incubation of a relatively large number of eggs. The resulting hatch yielded a larger number of poults than could be taken care of adequately. The history of this group is therefore of interest from a practical standpoint. The various broods which went into the large flock are given in Table III.

The ground covered by the enclosure and the surrounding acres had not been used for poultry for several years and had been ploughed and planted in the spring. A small flock of incubator chickens was permitted to mingle with the turkeys to some extent. The entire group of turkeys was herded on an adjoining tract of several acres under supervision from time to time and then returned to the enclosure. When the vegetation in the latter had been destroyed the fence was moved along to cover fresher ground. The maximum number together at any time was 52, in the middle of June. Withdrawals and deaths from blackhead, diseases other than blackhead, and accidents, and the killing of eleven for food in Nov., gradually reduced the total number to fifteen at the end of Nov.

Among the miscellaneous early causes of death were crowding and chilling in early June, leading to twenty deaths. Four died following unthriftiness and emaciation, two of impaction of the small intestine, due to eating coarse food, three with congestion of lungs, one with inflammation of the ceca, and one with impaction of the kidneys with urates. These losses, thirty-one in all, occurred up to June 15, and might have been largely averted if the accommodations had been adequate to prevent overcrowding.

About the middle of June withdrawals began to be made for experimental purposes. Miscellaneous causes of death continued operative, although on a much smaller scale. One died with ceca inflamed, one from congestion of lungs, and one from an undefined cause, late in June. Two were lost from undefined causes and one from unthriftiness, in July. Among the infectious diseases, aspergillosis appeared early in July. Two birds died of this disease; two were chloroformed and the foci discovered at autopsy.

Blackhead appeared first in June. In all, eight cases were discovered. The distribution in time was quite irregular, as shown in Table III. Thus one case occurred in June, one in Sept., one in Oct., three in Nov., and two in Dec. The remaining twelve were killed in Dec. and no lesions found in any.

Ten from this same lot, which had been removed to other enclosures during the season, were killed late in Nov. and early in Dec., and all found free from traces of blackhead lesions.

Experiments of 1919.

Experiment 9.—The fortuitous appearance of blackhead in enclosures protected in every way against the disease, except as it might be brought in by birds on the wing or small rodents, is well illustrated by the history of several flocks gradually merged into one larger flock during the summer of 1919.

The territory occupied during the season was an unused horse paddock enclosed by a high iron fence and not occupied by poultry for many years. The ground had been ploughed early in the spring and oats and grass sown. May 21. The first group of eight turkeys was moved to a brooder in the paddock when 9 days old. June 3. The brooder yard was enlarged to give more room. June 12. The brooder was replaced by a larger house. In the meantime one turkey had succumbed to blackhead on June 9. Others died on June 22, 29, July 2, 9, and 12. Two survived. To determine the infectious character of this flock and the soil, four additional young turkeys were introduced on June 30 (of which one died of softening of the bones soon afterward), three on Aug. 18; four on Sept. 5; and nineteen from another flock also on Sept. 5. In this last group, one had succumbed to blackhead on Aug. 13.

Sept. 5. The entire paddock was opened to the flock which now comprised thirty-one birds. Oct. 16. Twelve were taken out for a special feeding experiment. Oct. 20. One of the remaining nineteen died of blackhead. Nov. 17. Nine were taken out for another feeding test. Of the nine remaining, seven were

killed late in Dec. and all found free from lesions or scars of blackhead lesions. The ceca of all contained adult *Heterakis*, one bird carrying as many as twenty-nine specimens. The two left from the entire flock were penned with the older infected flock for breeding purposes. One of these died of acute blackhead on Jan. 30, 1920. Most of the data are brought together in Table IV.

The points of interest in the history of this flock are several. The unexpected, severe outbreak in June among birds still in the brooder resembles closely the occurrences so frequent upon farms where turkeys are raised. Thereafter only one other case occurred, making a mortality of seven among thirty-eight birds. The other feature of interest is the absence of immunity among the survivors, although all had been exposed at one time or another to a case. Of six fed subsequently with infectious material, all died. One of the original lot, of which two survived out of eight, died over 6 months later of the acute disease, after having been penned with older infected turkeys for over a month.

TABLE IV.

Lot No.	Date of hatching.	No. of ndividuals.	Introduced into enclosure.	Cases of blackhead.	Remarks.
	1919		*1919*		
1	May 12	8	May 21	One died on June 9, 22, 29, July 2, 9, 12.	June 3. Yard enlarged.
1	" 12	4	June 30	(One dies of softening of bones.)	
2	July 2	3	Aug. 18	One dies on Oct. 20.	
2	" 2	4	Sept. 5		Sept. 5. Entire paddock opened up to the thirty-one turkeys running together on Sept. 5.
3	May 27	19	" 5	None died. (One had died in this group on Aug. 13.)	

Experiment 10.—June 18. In another enclosure placed on land ploughed up and seeded to oats and grass in the spring, a flock of twenty-three incubator turkeys, hatched on June 10, was placed. June 24. Six were taken out for experimental uses, leaving seventeen. July 13. A turkey died, possibly of coccidiosis,

as oocysts were abundant. Blackhead appeared in the middle of Aug. and cases occurred well into Nov., as shown in Table V.

Although only three out of ten died, yet the symptoms of the rest and the presence of suspicious smooth, often deeply pigmented areas in one or both ceca and of whitish scars or foci in the liver make the diagnosis of blackhead fairly certain. Possibly the first case might be eliminated. This interpretation would push the beginning of the epidemic into Sept.

After counting out the dead and recovered cases, there were left six which had not shown signs of disease. They were killed late in Nov. The organs were free from lesions or scars. *Heterakis* was abundant in all instances. The extent

TABLE V.

Turkey No.	Beginning of illness.	Result.	Further observations.
	1919		
332	Aug. 19	Recovered.	Nov. 22. Killed. Normal.
354	Sept. 9	"	" 25. " Some scars in liver and smooth areas in ceca.
355	" 13	"	Reserved for breeding.
356	" 28	"	Jan. 21, 1920. Killed. Scars in liver and smooth and dark pigmented areas in ceca.
357	" 30	"	Nov. 24. Killed. Some whitish spots on liver and pigmented areas in one cecum.
358	" 30	Oct. 24. Died.	Blackhead.
359	Oct. 4	" 24. "	"
363	" 21	Nov. 13. "	"
364	Nov. 1	Recovered.	Nov. 22. Killed. Whitish foci in liver and pigmented areas in ceca.
365	" 6	"	Reserved.

of the disease may perhaps be accounted for by the fact that this flock was allowed to run over an adjoining part of the land on pleasant days, in the care of an attendant who drove them back into the smaller enclosure after 1 or 2 hours.

Experiment 11.—In this experiment a spontaneous outbreak among turkeys penned with incubator chickens occurred. The enclosure was placed on land which had been ploughed up and sown to grass and oats in the spring.

There were nineteen chickens in the flock. July 28. Two young turkeys were penned with them. Both became ill in 23 and 26 days respectively. One was chloroformed and the diagnosis of blackhead confirmed. Young stages of *Heterakis* were present. The other recovered and was killed in Nov. The liver showed healed foci and there were found a constriction of one cecum due to scar tissue and smooth areas in both ceca. Sept. 5. Three additional turkeys were placed in this group. All three contracted blackhead, one after 31 and the others after

34 days. One died. Mature *Heterakis* were found in the ceca. The others recovered. Both were killed in Jan., 1920, and in each there were changes indicative of healed blackhead lesions. The chickens remained clinically well. Eight were killed early in Oct. and two late in Nov. No lesions were detected. *Heterakis papillosa* was present in the ceca.

This group is of interest from the fact that all five turkeys placed with the chickens contracted blackhead. The relatively short period of incubation may have been due to the comparatively late exposure, since all experiments have pointed to an accumulation of disease-producing factors with the advance of the summer. The bearing of the chickens on the incidence of blackhead is not clear. It seems as if they may have picked up the virus with *Heterakis* and cultivated it with the latter in the ceca.

Experiment 12.—This experiment was designed to test the infectiousness of an enclosure recently occupied by a group of older turkeys as compared with the infectiousness of the same group on fresh soil.

(*a*) *Exposure to Older Turkeys on New Ground.*—The flock consisted of seven hens and a male, hatched during 1917 and 1918. June 2. They were cleansed and all soil was washed from the feet and they were then placed on grounds not heretofore used for poultry. The coops and nests were thoroughly scrubbed and cleansed before they were moved to the new grounds. June 3. Six young turkeys 7 days old were transferred in a brooder to the same new grounds and allowed to run out during warm weather. During cold or rainy days they were kept in the large coops occupied by the older turkeys at night to increase opportunities for infection. June 16. They were permitted to run freely with the older birds. In the meantime one had been smothered by the others. The remaining five did well up to Aug. 6, 64 days after the beginning of the exposure, when one became sick. One became sick on Aug. 7, one on Aug. 10, and two others on Aug. 15. One was killed on Aug. 20 and the diagnosis of blackhead confirmed. Some young and nearly adult *Heterakis* were found in the ceca, but no coccidia. The four remaining turkeys gradually recovered and all were well by the end of Aug. Evidence that they had passed through an attack was furnished when they were killed, two late in Dec. and two towards the end of Jan., 1920. Constrictions, obliterations of the longitudinal folds and pigmentation in the ceca, and scars or grayish foci in the liver were present.

In the group of twenty-two turkeys from which the above were taken one case occurred on Aug. 4. On Sept. 5 this group was merged with another group and one other case occurred in the combined flocks during the remainder of the season.

Aug. 23. The experiment was continued by placing three fresh turkeys into the enclosure with the older turkeys. The concentration of infectious material which had been going on was shown by the rapidity with which this new lot be-

came affected. One was ill on Sept. 4 and died on Sept. 12. The second was ill on Sept. 6 and it was killed on Sept. 9 and found diseased. Individuals of *Heterakis* were present in an immature stage in both cases. The third bird showed signs of illness on Nov. 17, but recovered. When killed on Jan. 22, 1920, the only indication of former disease was a large white healed focus in the liver. *Heterakis papillosa* was present.

(*b*) *Exposure on Grounds Vacated by Older Turkeys.*—The grounds occupied by the older flock during the winter were cleared, as stated under (*a*), on June 2, and remained vacant for 22 days. June 24. Six turkeys 2 weeks old were placed in it. 30 days later a young turkey showed signs of drooping and died of blackhead 15 days later. Three others contracted the disease, two on July 29 and one on Aug. 5. In all, both ceca were diseased. In two a few coccidia were found and in two larval nematodes. The fifth became sick on Sept. 27 and recovered. When killed late in Jan., 1920, there were a number of large scars in the liver and a smooth patch in each cecum. Nov. 2. The last of the six, an exceptionally vigorous bird, showed signs of disease and died 3 days later of blackhead.

Meanwhile, the loss of the birds was partly made good by introducing four young healthy turkeys on Aug. 18, when they were 47 days old. These also contracted blackhead. One died on Sept. 25, one on Oct. 20, and one on Oct. 21. The fourth showed signs of disease on Oct. 4, but recovered. It was killed on Jan. 22, 1920. The liver showed a few whitish foci and each cecum a smooth area on the mucosa.

In this experiment, the intensity of the infectious agent seems to have been nearly equal in the two enclosures. Disease appeared 30 days after the beginning of the exposure, in the vacated enclosure, and 64 days in the new enclosure containing the older birds. There is evidence that the infection became, if anything, more rather than less concentrated as the season progressed. This was indicated by the shorter time elapsing between exposure and disease.

The fact that 100 per cent of the exposed birds contracted the disease neutralizes the possible error involved in using birds from flocks in which spontaneous disease occurred. The percentage of such cases in the stock flocks was low and the disease first made its appearance some time after it had begun in the experimental enclosures.

One flock which consisted originally of twenty-three incubator turkeys, hatched on July 2, deserves mention here since blackhead did not appear in the enclosure during the greater part of the summer season. They were placed on a plot of ground on July 4, and allowed to run in a small yard on July 8 and in a larger enclosure on July 23. The flock was gradually reduced by withdrawals, four on

August 7, four on August 18, and three on August 23. Some died of non-infectious troubles. Seven were still present on September 5. These were later used in other experiments. They had thus been on the soil 59 days without any symptoms of blackhead.

During October a mild form of chicken-pox appeared in all but one of the groups of turkeys. It was first noticed among the turkeys in Experiment 9, and later in Experiments 12, *b*, 10, and 12, *a*. The mode of introduction remains unknown. This epizootic had no appreciable influence on the health of the birds.

SUMMARY.

The foregoing experiments in outdoor, unprotected enclosures demonstrate the difficulties surrounding the rearing of turkeys. These are discussed from another view-point[2] and to avoid repetition only a few outlying facts should be considered here.

The occasional presence of coccidia, the presence of *Heterakis papillosa* in the ceca, the occurrence of cases of aspergillosis and of chicken-pox in incubator-bred birds which did not come in contact with other domesticated birds, except in a few cases with incubator-bred chickens, show clearly that turkeys are picking up from the ground material deposited by other birds. The agent of blackhead must come from the same sources.

The field experiments show a steadily increasing concentration of the infection from 1917 to 1919, even though the ground had been ploughed and seeded before use. As a result, the various groups of turkeys became infected to a greater degree. The growth in the intensity of the disease may be in part ascribed to an accumulation on the soil of infectious agents during any given season after they had been introduced, but it is hardly acceptable as an explanation from season to season, when the soil was either virgin, as regards poultry yards, or ploughed deep and seeded before use. A more rational hypothesis is the gradual attraction of birds in larger numbers and greater variety on account of the food supply in the turkey enclosures and the more intensive cultivation of the land surrounding the laboratory and animal buildings since the beginning of the experiments in 1917.

[2] Graybill, H. W., and Smith, T., *J. Exp. Med.*, 1920, xxxi, 647.

The intensity of the outbreaks due to the confining of young turkeys with birds over a year old which had been infected during the preceding year, or on grounds previously occupied by them, was in all instances much greater than in the spontaneous outbreaks. The cases amounted to nearly 100 per cent of the exposed. On the other hand, the number of cases in the control flocks varied and was very low in some groups. It could have been kept down if the sick birds had been promptly removed and not permitted to recover on the same ground. However, the object of the experiment was not to suppress the disease, but to see to what extent it would develop.

It is self-evident that the results obtained apply strictly only to that part of the country where the experiments were made. We have at present no means of knowing whether the sources of infection would become more numerous and concentrated with a higher mean annual temperature, or the reverse. Only by using incubator turkeys exclusively for such tests and eliminating the older turkeys and domesticated birds as carriers, can the miscellaneous, at present not controllable sources of the agents of this disease in different localities and the chances of successful rearing be determined.

PRODUCTION OF FATAL BLACKHEAD IN TURKEYS BY FEEDING EMBRYONATED EGGS OF HETERAKIS PAPILLOSA.

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Since the protozoan agent of blackhead was described and its relation to the lesions of the ceca and liver defined by one of the authors[1] in 1895, very little concerning the mode of transmission of the agent or the conditions favoring its invasion and multiplication has come to light. In 1895 Moore[2] fed the liver and ceca of three diseased turkeys to two healthy ones. The days of feeding were Nov. 28, Dec. 3, and Jan. 3. On Jan. 11, 44 days after the first feeding and 8 days after the last feeding, one of the turkeys died of blackhead. In another experiment beginning Nov. 28, Moore penned four healthy turkeys with two diseased ones. During the night for a period of about 2 weeks the feces of the sick ones were collected and fed, mixed with the feed, to the well ones on the following day. One of the two sick turkeys died in 3 days and the other survived. Of the four which received the discharges and were also exposed during the day to the sick ones and their environment, three became diseased. Two died in 22 and 27 days respectively; the third bird, well when killed 46 days after the beginning of the experiment, showed extensive lesions of blackhead.

In Sept., 1913, one of the authors[3] fed two incubator turkeys, hatched early in June of the same year and reared on virgin soil, the chopped up diseased ceca of two turkeys 2 to 3 months old. Both remained clinically well up to Dec. 24 when one was stolen. An autopsy on the other 3 days later showed it to be normal.

New Experiments in Feeding Feces.

During the years 1918 and 1919 certain attempts to induce blackhead by feeding feces of older turkeys which had successfully passed through exposure to disease in the preceding year were made. These

[1] Smith, T., *U. S. Dept. Agric., Bureau Animal Industry, Bull. 8*, 1895, 7.
[2] Moore, V. A., *U. S. Dept. Agric., Bureau Animal Industry, Circular 5*, 1896, 1.
[3] Smith, T., *J. Med. Research*, 1915, xxxiii, 243.

attempts, although leading uniformly to negative results, are detailed below because of their bearing on subsequent more successful trials. It should be stated here that cohabitation in the same enclosure with the turkeys furnishing the feces produced blackhead in a certain number of young turkeys.

Experiment 1.—June 10, 1918. A male bird, 2 years old, was confined in an isolation unit after all soil had been washed from its feet. The feces were collected and fed to four young turkeys confined in a cage in the same unit. The turkeys were 30 days old. The feces were mixed with the food and fed on June 11, 12, 13, and 14. The experiment was closed on July 9 and the turkeys were released. At no time during a period of 28 days did they show symptoms of blackhead. These turkeys were used in other experiments during the season but none of them developed blackhead. One died on July 22 of a disease of the bones, resulting in all probability from confinement in the cage. The others were killed at the close of the year, one on Dec. 10 and the others on Dec. 20. The organs were uniformly normal.

Experiment 2.—Three turkeys, hatched on May 12, 1918, were fed feces of two old turkeys under the following conditions: The feces were collected on Sept. 2 and 3. A portion was fed on Sept. 2 with the food, morning, noon, and night. The remainder was placed in Petri dishes at room temperature until fed. The later feedings took place on Sept. 6, 9, and 13.

The young turkeys had been running with some chickens in a special enclosure and were kept in it except on the dates of the feeding. The feeding was done in a cage. They remained well and when two were killed late in Dec. no lesions were found. The older turkeys from which the feces were obtained belonged to an enclosure in which young had contracted blackhead. It will be noted that in this trial an incubation of the feces at room temperature for 3, 6, and 10 days respectively failed to make the test positive.

Experiment 3.—The feces of adult turkeys were fed to chickens.

In view of Milks'[4] observations on the occurrence of blackhead in young chickens, it was believed that very young chickens would be suitable for feeding experiments, and such an experiment was undertaken in the spring of 1919, before young turkeys were available. This experiment was conducted in a brooder and was begun on Apr. 4, when the chicks were 8 days old. Six chicks were used, two of each of the following breeds: Rhode Island Red, White Leghorn, and Barred Plymouth Rock. The feces were collected as fresh as possible from the coops occupied by the old flock kept for breeding purposes. It consisted of eight birds, and in the main contained the same ones that were in it the previous year. Feces having different physical characters were collected. Those not fed fresh were kept in Petri dishes at room temperature. In all, from Apr. 4

[4] Milks, H. J., *Louisiana Agric. Exp. Station, Bull. 108*, 1908, 1.

to 17, feces were fed on 12 days. They ranged in age, counting from the date of collection, from less than 1 to 5 days. The chicks at no time showed symptoms of blackhead. One showed lameness due in all probability to confinement.

The manner of feeding the feces was then modified to reproduce more nearly natural conditions. On Apr. 17 a galvanized iron tray was placed in the outer compartment of the brooder and filled with sterilized soil. Feces were placed from time to time on the soil and the feed was always placed there. The soil was kept moist. Fresh and 1 day old feces were placed on the tray on Apr. 17 and on Apr. 18, 19, 20, 22, 23, 24, and 25 fresh feces were placed there.

Aside from lameness the chicks showed no other symptoms. Three of them were chloroformed on May 2, and three on May 6, 28 and 32 days respectively after the beginning of the experiment, and all were found free from blackhead.

After it had been found that by placing young turkeys with the old flock, subsequent to removal to new soil, they contracted blackhead, it was decided to undertake still another experiment feeding the feces of the old flock to young turkeys.

Experiment 4.—The turkeys of the old infected flock were removed by twos and placed in an indoor unit to facilitate collecting feces of all alike. The feces not fed fresh were kept in Petri dishes at room temperature. Beginning on Aug. 7, four young turkeys 36 days old, two white and two bronze, were fed in a cage. The feeding was carried out daily, with the exception of Sunday, to Aug. 19. After the feeding they were still kept indoors, but in a pen to enable them to be more active. The feces from eight older birds were used. When fed, they were either fresh or up to 8 days old. On Sept. 5 the young turkeys were removed to an outdoor enclosure. 23 days after the last feeding the experiment was closed. None had shown signs of disease in the meantime. Owing to a shortage of incubator turkeys these were used again in later experiments.

Feeding Experiments with Embryonated Eggs of Heterakis papillosa.

In experiments conducted in 1914,[3] 1916,[5] and since that time, relating to the mode of transmission of the disease, it appeared that young diseased turkeys which did not recover were incapable of transmitting blackhead to healthy ones penned with them. In addition to this, it became evident that soil recently occupied by old turkeys was infectious to young ones.[6] These observations along with negative results in feeding feces suggested the existence of some

[5] Smith, T., *J. Exp. Med.*, 1917, xxv, 405.

[6] Smith, T., and Graybill, H. W., *J. Exp. Med.*, 1920, xxxi, 633.

additional factor or factors in the transmission of blackhead. Previous experience and experiments gave no direct clue as to the nature of such a factor. There were a number of hypotheses which might fit the conditions observed or at least not be contradicted by them. The protozoan parasite invading the walls of the ceca and the liver evidently multiplied very rapidly when once started. It was also evident that this multiplication came to an abrupt stop and that the parasites in the tissues perished rapidly owing to an acquired immunity of the host or some other factor operating against the invading parasite at the height of the invasion. It was assumed that this parasite might be aided by some lesion or injury of the mucosa of the ceca to enter the tissues and multiply there, since the invasion in itself did not seem to be a normal part of the life cycle of the parasite. There was no evidence of the formation of any resistant stages in the tissues or of the normal discharge of the parasite outward, as, for instance, in coccidiosis. If the parasite was not to be regarded as wholly aberrant, whatever normal cycle there was seemed to belong to the lumina of the ceca. In harmony with this hypothesis a nematode parasite, *Heterakis papillosa*, occurring in the ceca of turkeys, chickens, and some other birds, was brought into the experiment as the possible associated factor.

Experiment 5.—This experiment consisted in feeding to young turkeys embryonated eggs of *Heterakis papillosa* and feces from adult turkeys. Worms were collected from the ceca of healthy chickens, killed for this purpose, by washing and sedimenting the contents and picking out the worms. These were cut up in a small quantity of normal salt solution to liberate the ova. The suspension was kept in Petri dishes at room temperature and distilled water added from time to time to make up for evaporation.

After the cultures had been kept for 17 days, examination showed that the ova contained living embryos. On Sept. 11 they were added to the feed of two turkeys which had been used in the preceding experiment. To ensure the complete ingestion of the mixture the turkeys were placed in a cage. On the same and the 2 following days feces from two older turkeys, collected on Sept. 10 and 11 were fed to the same birds. They were then placed with two other turkeys, also from the preceding experiment, which were to act as controls, in an outdoor enclosure.

Both infected turkeys became sick on Sept. 26; *i.e.*, 15 days after the feeding of ova. One died of blackhead after 3 days, the other after 6 days. The two control birds remained well and 43 days after the beginning of the experiment they were used in Experiment 7.

Experiment 6.—In this experiment the two factors, embryonated eggs of *Heterakis papillosa* and feces of adult turkeys, were kept distinct.

The young turkeys used in this experiment were from a group consisting of portions of three different broods of incubator turkeys, numbering in all about thirty individuals.[6] They had been running together for some time in a large enclosure. In some unknown way the parasite of blackhead had been introduced into this enclosure and six out of eight turkeys in one brood died of this disease during June and the first few days of July before the broods were mingled. Following these cases one turkey died of blackhead on Aug. 13 and another on Oct. 20. *Heterakis papillosa* was known to be present from some of the autopsies made. The latter was the only case that appeared in the remainder of the flock after the birds chosen for this experiment had been removed. Of the turkeys used Nos. 308, 309, and 312 were hatched on May 12, 1919, Nos. 360, 361, and 362 were hatched on May 27, and Nos. 345, 346, 350, 351, 352, and 353 on July 2. The turkeys were separated into four groups on Oct. 16: Nos. 309, 312, and 352 (controls) were fed nothing. Nos. 346, 350, and 360 received embryonated eggs plus turkey feces. Nos. 345, 353, and 361 received embryonated eggs only. Nos. 308, 351, and 362 received turkey feces only.

The *Heterakis* eggs used in this experiment were obtained from worms collected from the ceca of four chickens on Oct. 1, and were prepared for culture and incubated as in Experiment 5. The feces were obtained from two older turkeys of the infected flock, the same ones that were used in the preceding experiment, and were less than 48 hours old when fed.

The three feedings of ova and feces, of ova, and of feces, respectively, were conducted in separate enclosures and the three groups of turkeys were brought together after the feeding in a new enclosure with the controls, care being taken to remove all traces of soil from the feet by washing them thoroughly before the groups were finally assembled.

The result of the experiment is briefly told. Of the group which was fed eggs plus feces, Nos. 346 and 360 showed symptoms on Oct. 28, and No. 350 on Oct. 29; *i.e.*, in 12, 12, and 13 days respectively. They were killed and autopsied a few days later and the diagnosis was confirmed by the lesions in ceca and liver. Of the group fed ova only, Nos. 345 and 361 showed symptoms on Oct. 27, and No. 353 on Oct. 29; *i.e.*, in 11, 11, and 13 days respectively. Several days later they were killed and the autopsy showed marked lesions of ceca and liver.

The three that were fed feces only showed no symptoms of disease and were killed and autopsied, two on Nov. 10, and one on Nov. 11; *i.e.*, about 2 weeks after all those fed eggs had become sick. No lesions were found in these birds. A careful search for *Heterakis* in the cecal contents showed the presence of one mature male in No. 308, eight mature males in No. 362, and four females, two males, and one larval nematode in No. 351.

The three control birds showed no symptoms of disease and were killed on Nov. 10 and 11. One of these (No. 352) had lesions of blackhead; the others were entirely normal. In the ceca of No. 309, two mature males and one female, and in

No. 312, one female *Heterakis* were found. In the affected control both ceca showed many elevated indurations up to 0.5 cm. in diameter. The largest had a central superficial slough or scab. In the liver only a few small whitish foci, 1 to 2 mm. in diameter, and one focus of congestion were found. In fresh preparations from the ceca a few *Heterakis* eggs and one larval worm were found. In the washed and sedimented feces two mature and two immature females of *Heterakis* and many larval worms with attenuated posterior extremity and a bulbous esophagus were found. The mouth was without buccal capsule and provided with four papillæ.

The lesions of this case suggest that the disease may have been produced by something that occurred after the beginning of the experiment. This bird may have ingested ova from the adult worms in the other birds or perhaps embryonated eggs passing through the intestines of the others soon after they had been fed. In any case, the causes bringing about disease in this instance will probably be cleared up in future experiments when the external sources of the two factors, *Amœba meleagridis* and *Heterakis papillosa*, shall have been more definitely located.

Experiment 7.—This test may be regarded in a way as supplementary to Experiment 5. The two controls there surviving were fed 43 days after the beginning of Experiment 5 with embryonated eggs of *Heterakis papillosa*. The worms were collected from four chickens and the ova incubated as heretofore for 16 days, when they were fed mixed with the daily ration. The feeding was done on Oct. 24. One turkey became ill on Nov. 4, the other on Nov. 6; *i.e.*, 11 and 13 days after feeding. They were chloroformed and the diagnosis was confirmed by finding lesions of ceca and liver.

Experiment 8.—Owing to the lateness of the season, only one additional test was undertaken. This consisted in feeding the contents of the ceca of turkeys affected with blackhead which had died or had been chloroformed and the feces of older turkeys, both incubated for 15 days, but free from *Heterakis* eggs.

The contents of the ceca of four diseased turkeys were suspended in normal salt solution and passed through a No. 40 wire mesh. The strained suspension was placed in Petri dishes in shallow layers. The incubation began on Nov. 3. Careful examination of the fluid under a low power failed to show the presence of ova.

Nov. 3. The feces of older healthy turkeys were collected, suspended in water, and passed successively through a tea strainer and wire screens of Nos. 40, 60, 100, and 200 mesh respectively. The final sediment was washed twice in normal salt solution and distributed into four Petri dishes. Nov. 14. The incubated dishes were carefully examined under a low power. Two *Trichosoma* ova but no others were detected.

Nov. 18. Nine turkeys, taken from the group from which those in Experiment 6 came, were selected. Three were reserved as controls, three fed cultures from diseased turkeys, and three cultures from older turkeys. After the feeding the three groups were brought together in one outdoor enclosure. No symptoms of disease appeared, and the birds were killed and autopsied as shown in Table I.

It will be noted that the turkeys were killed 21 and 29 days after the experiment had been begun. In the collection of the worms the contents of the ceca were washed and sedimented. Lesions were uniformly absent.

TABLE I.

Turkey No.	Date killed.	No. of *Heterakis* individuals.
	1919	
310	Dec. 9	1 adult.
366	" 17	8 adults.
367	" 17	14 "
368	" 9	15 "
369	" 17	15 "
370	" 17	10 "
371	" 17	19 "
372	" 17	8 "
373	" 9	6 " and 2 young males.

DISCUSSION AND SUMMARY.

In four experiments, three with young incubator turkeys and one with young incubator chickens, in which the feces of old turkeys from an infectious flock, kept at room temperature up to 5, 8, and 10 days, were fed, no infection resulted.

In an experiment in which two of four young incubator turkeys used in one of the above experiments were fed embryonated eggs of *Heterakis papillosa* and feces of turkeys from an infectious flock both contracted blackhead. Two controls remained well. Later they were fed embryonated eggs of *Heterakis papillosa* and both contracted blackhead.

In another experiment three incubator turkeys received embryonated eggs plus turkey feces from an infectious flock. All contracted blackhead. Three received embryonated eggs alone; all contracted blackhead. Three received turkey feces only; none contracted blackhead. Three controls received nothing; one showed blackhead lesions at the autopsy.

In a final experiment three turkeys were fed cultures of feces from the ceca of diseased turkeys, three were fed cultures of feces of old turkeys from an infected flock, and three controls were fed nothing. None contracted blackhead. The cultures of feces were prepared

precisely as were the earlier ones containing *Heterakis* eggs but without the latter.

From these experiments it becomes evident that blackhead may be produced in healthy incubator-raised turkeys, reared in the open in an environment where blackhead occurs, but out of direct contact with old turkeys and other poultry, by feeding cultures of embryonated eggs of *Heterakis papillosa*, prepared by cutting up the worms in isotonic salt solution and incubating the suspension at room temperature.

These very definite and clear-cut results outweigh any objections which may be raised against the use of turkeys which had been in earlier experiments and which came through such experiments without any signs of disease, or which came from control flocks in which spontaneous cases had occurred. The short time elapsing between feeding embryonated eggs and the first signs of disease made these experiments unusually impressive. It should be stated, furthermore, that from a precise individual record of all turkeys it was possible to select birds from control flocks in which the infection had either not appeared or was very low. All but two turkeys in flocks serving as sources of this material were killed at the close of the year. None at any time had shown symptoms of disease, and no scars or other abnormalities of ceca and liver were found. Furthermore, all other control birds and those in field experiments, with the exception of two reserved for breeding, were likewise killed. As a result of these autopsies, it was determined that of all birds in which symptoms of disease had not been recorded during life, none showed abnormalities or scars at autopsy. The protozoan factor in blackhead was probably disseminated when the first spontaneous cases occurred in the stock, unless it was present and made invasive by incubation in the cultures fed. This latter theory seems at present not acceptable because of the wholly negative outcome of Experiment 8.

The production of acute blackhead by feeding embryonated eggs to turkeys in whose ceca adults of *Heterakis papillosa* are already present seems incomprehensible at first thought. A tentative explanation to be offered is that the worms when invading the ceca in large numbers break down the resistance of the bird which is able to protect itself against a few. This may account for the very irregular

occurrence of cases in contact with older recovered birds on infected grounds. The rôle of *Heterakis* as a preliminary agent may also account for the continuing high mortality in turkeys in which the disease has been operating for so many generations to eliminate the most susceptible. It now seems highly probable that the turkey has become relatively resistant to the invasion of the protozoan parasite acting alone and that such invasion may require other agencies. Whether *Heterakis papillosa* is the only, or at any rate, the chief accessory agent or whether there are others, living or inert, which when ingested by the turkey assist in preparing the way for the destructive invasion of the walls of the ceca and the liver by *Amœba meleagridis* is a question now open to solution by experimentation.

The relation of common poultry to outbreaks of blackhead may be accounted for, at least in part, by the fact that they are hosts of *Heterakis papillosa*. How frequently they also carry *Amœba meleagridis* remains to be determined.

Since earlier communications have contained certain practical suggestions on the rearing of turkeys and the prevention of blackhead, it is not out of place here to point out that the additional information presented in this article simply emphasizes the suggestions already made. Turkeys should be raised in the incubator and brooder and kept away from older turkeys and poultry. The shelters should be moved from time to time to prevent a too concentrated infection of the soil with *Heterakis* ova. Inasmuch as the factors producing blackhead may be deposited by certain still undetermined birds on the wing, disease may be looked for at any time during the warm season. It is not, however, very readily transmitted, and in the experiments described elsewhere[6] the mortality from spontaneous blackhead was low. The flock should be looked over as frequently as possible, and whenever a turkey begins to droop, it should be isolated and killed if the drooping continues over several days. If such turkeys are allowed to recover, they should not be returned to the young flock but kept with older, presumably infected birds. Such birds are entirely satisfactory as a source of eggs, since there is no evidence that the latter transmit the infection.